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Nuclear Matrix Proteins Distinguish Normal Diploid Osteoblasts from Osteosarcoma Cells¹

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Abstract

Interrelationships between nuclear architecture and gene expression were examined by comparing the representation of nuclear matrix proteins in ROS 17/2.8 rat and MG-63 human osteosarcoma cells with those in normal diploid osteoblasts. The tumor-derived cells coexpress genes which are expressed in a sequential and mutually exclusive manner during the progressive stages of osteoblast differentiation. In osteosarcoma cells two-dimensional electrophoretic analysis indicates a composite representation of nuclear matrix proteins characteristic of both the proliferative and postproliferative periods of osteoblast phenotype development. In addition, nuclear matrix proteins unique to the tumor cells and the absence of nuclear matrix proteins found only in normal diploid osteoblasts are observed. Tumor-specific nuclear matrix proteins include those expressed in a proliferation-dependent and independent manner. There is a parallel relationship between nuclear matrix proteins and the expression of cell growth and tissue-specific genes during osteoblast differentiation and in osteosarcoma cells where the developmental sequence of gene expression has been abrogated. Nuclear matrix proteins therefore provide markers reflecting defined periods of bone cell differentiation and phenotypic characteristics of an osteosarcoma.

Introduction

Transformed and tumor cells exhibit aberrations in the expression of cell growth and tissue-specific genes during both the onset and progression of neoplasia. A fundamental area of investigation is that of tumor-related modifications in regulatory parameters that selectively restrict or render developmentally expressed genes competent for transcription.

Primary cultures of normal diploid osteoblasts undergo a sequential expression of cell growth and tissue-specific genes that are functionally related to development of the bone cell phenotype (reviewed in Refs. 1, 2–5). This developmental sequence initially includes the expression of genes that support cell growth and biosynthesis of the type 1 collagen bone extracellular matrix. Expression of genes associated with extracellular matrix maturation, organization, and mineralization are confined to the subsequent postproliferative periods of osteoblast differentiation. In contrast, osteosarcoma cells such as the ROS 17/2.8 line exhibit loss of the developmental sequence of gene expression characteristic of normal diploid osteoblasts (6, 7). Abrogation of stringent growth control in these rodent osteosarcoma cells is accompanied by and potentially functionally related to concomitant expression of genes involved with both proliferation and phenotypic properties of mature bone cells.

The nuclear matrix, the nonchromatin nuclear substructure organized as a proteinaceous network of polymorphic anastomosing fibers, may contribute to the transcriptional regulation of gene expression

during osteoblast differentiation and modifications in the expression of cell growth and tissue-specific genes observed in osteosarcoma cells. The linkage of nuclear matrix composition to cell and tissue type in general is consistent with nuclear matrix participation in the regulation of gene expression (8, 9–18). Nuclear matrix composition has also been shown to be uniquely characteristic of specific tumor cells (15, 19–21, 22). Other evidence supporting nuclear matrix involvement in the regulation of gene expression includes the preferential association of actively transcribed genes with the nuclear matrix (23–26), the localization of RNA synthesis and pre-mRNA splicing on the nuclear matrix (16, 27–33), and the presence of some but not all steroid receptors in the nuclear matrix fraction (34–37).

A role for the nuclear matrix and transcriptional regulation during progressive development of the osteoblast phenotype is supported by nuclear matrix proteins characteristic of each of the three principal periods of osteoblast differentiation (38). More direct evidence for nuclear matrix proteins contributing to control of osteoblast differentiation is provided by developmentally mediated association with the nuclear matrix of transcription factors which are cell growth related (24), broadly represented (39), and bone specific (40). Proliferation-dependent partitioning of oncogene encoded transcription factors between the nuclear matrix and nonmatrix nuclear fraction has been observed in osteoblastic cells (39).

To experimentally address interrelationships between nuclear architecture and gene expression in bone tumor cells we compared nuclear matrix proteins of normal diploid osteoblasts during differentiation with those in ROS 17/2.8 osteosarcoma cells. Consistent with coexpression of genes in osteosarcoma cells which are sequentially and independently expressed during osteoblast differentiation, we observed a composite representation of nuclear matrix proteins in ROS 17/2.8 cells which are characteristic of both the proliferative and postproliferative periods of the osteoblast developmental sequence. We identified proliferation-dependent and growth-independent nuclear matrix proteins that are present in rat as well as human osteosarcoma cells but absent in normal diploid osteoblasts. Expression of other nuclear matrix proteins is restricted to normal diploid bone cells.

Materials and Methods

Cell Culture. Primary cultures of normal diploid osteoblasts were obtained from the calvaria of timed pregnant rats (Sprague-Dawley). The isolation and culture of primary osteoblasts were as described previously (1). Ros 17/2.8 osteosarcoma cells (41) (generously provided by Drs. Sevgi Rodan and Gideon Rodan, Merck Sharp & Dohme, West Point, PA) were grown in F12 media supplemented with 5% horse serum.

Nuclear Matrix Protein Isolation. Cells were pulse-labeled with 25 $\mu\text{Ci/ml}$ of L-[³⁵S]methionine/ml in methionine-free minimum essential medium for 2 h at 37°C followed by the isolation of nuclear matrix proteins according to the protocol of Fey et al. (8). Two-dimensional gel electrophoresis was performed according to the method of O'Farrell (42).

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Northern Blot Analysis. Extraction of total RNA from cell pellets and hybridization conditions with a panel of probes was as detailed elsewhere (43).

Results and Discussion

Coexpression of Growth-dependent and Postproliferative Tissue-specific Genes in Osteosarcoma Cells. It has been well established that differentiation of proliferating, normal diploid osteoblasts to postproliferative cells in a mineralized type 1 collagen extracellular matrix is associated with a three-stage developmental sequence of gene expression which supports the progressive establishment of bone tissue organization (1). This developmental expression of cell growth and tissue-specific genes has been observed in primary cultures of normal diploid mammalian osteoblasts during the formation of nodules of multilayered osteocytic cells in a mineralized extracellular matrix with a bone tissue-like organization, as well as during osteoblast differentiation *in vivo* (reviewed in Refs. 2–5 and 7). First, proliferating osteoblasts express genes which support cell cycle and cell growth control (*e.g.*, histone, see *c-myc*, *c-Fos* and *c-Jun*) together with genes for the initial biosynthesis of the bone extracellular matrix (*e.g.*, transforming growth factor β , type 1 collagen, fibronectin). Equally important is the fact that regulatory mechanisms are operative at this time which suppress the expression of genes associated with mature osteoblast phenotypic properties. During a second developmental period immediately following the down-regulation of proliferation, genes involved with the continued biosynthesis, maturation, and organization of the bone extracellular matrix are expressed. Then during a third developmental period, gene expression associated with competency for the ordered deposition of mineral occurs.

As indicated in Fig. 1 osteosarcoma cells coexpress genes that are sequentially expressed in a strict developmentally dependent manner in normal diploid osteoblasts. For example, in rat osteoblasts, a com-

parison of cellular mRNA levels for cell growth and bone tissue-related genes during the proliferative and postproliferative period of osteoblast differentiation indicates that histone gene expression is confined to the initial proliferation period while alkaline phosphatase, osteopontin, and osteocalcin expression occur postproliferatively. In ROS 17/2.8 osteosarcoma cells, coexpression of cell growth and tissue-specific genes is strikingly illustrated by high levels of gene expression in proliferating cells that occurs in normal diploid cells only during the immediate postproliferative period of extracellular matrix maturation. Fig. 1 shows that alkaline phosphatase is expressed at 80% of maximal level in proliferating ROS 17/2.8 osteosarcoma cells. Also apparent is the lower but significant expression of genes associated with the mature normal diploid osteoblast undergoing extracellular matrix mineralization (*e.g.*, osteocalcin and osteopontin).

It therefore appears that in osteosarcoma cells regulatory mechanisms which support the ordered expression of genes throughout the development of the osteoblast phenotype are operative irrespective of the level of ongoing proliferation, reflecting an apparent compromise of proliferation/differentiation interrelationships. In addition there are modifications in regulatory mechanisms that normally are involved with changes in gene expression at the developmental transition point in mature osteoblasts when the immediate postproliferative genes exhibit decreased expression (*e.g.*, alkaline phosphatase) and genes associated with the onset of extracellular mineralization are up-regulated (*e.g.*, osteocalcin and osteopontin).

Composite Representation of Developmentally Regulated Nuclear Matrix Proteins in Osteosarcoma Cells. Support for participation of the nuclear matrix in transcriptional control of osteoblast genes is provided by the two-dimensional electrophoretic profiles of nuclear matrix proteins analyzed throughout the osteoblast developmental sequence. Changes in the protein composition of the nuclear

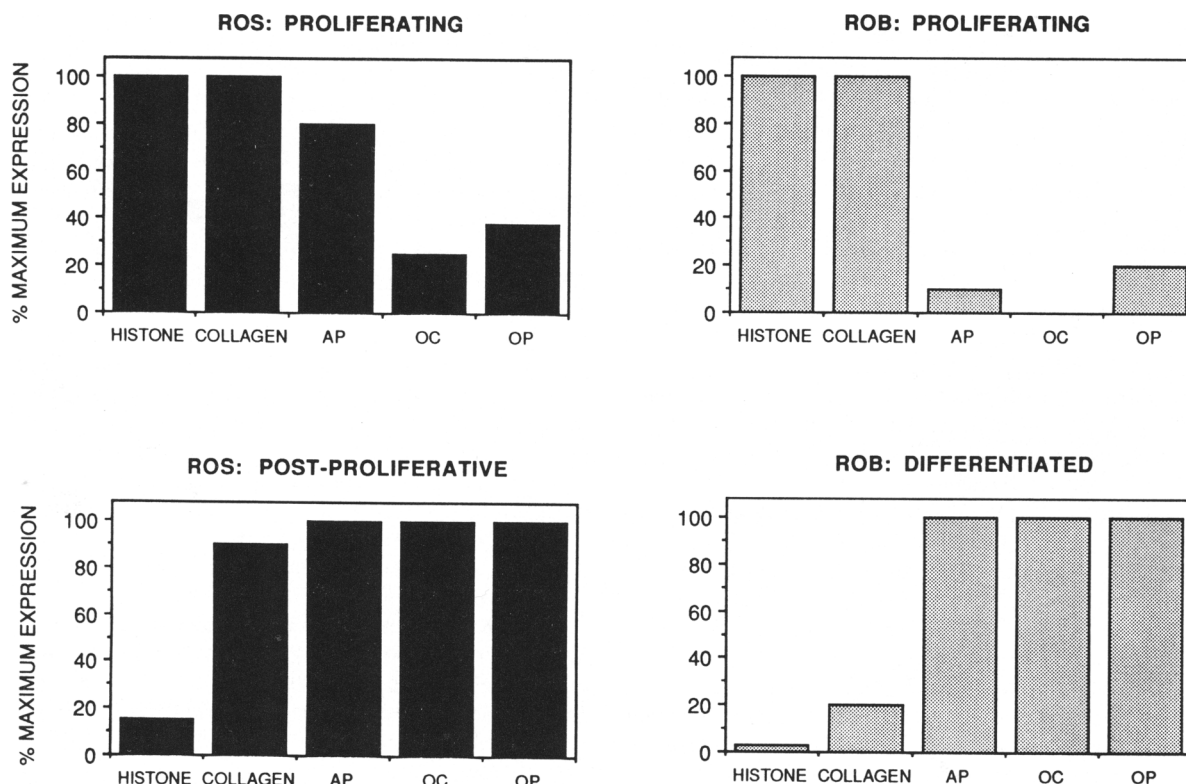


Fig. 1. Comparison of gene expression in proliferating and postproliferative normal diploid osteoblasts with those in osteosarcoma cells. Expression of representative cell growth (histone) and bone related [type 1 collagen, alkaline phosphatase (AP), osteopontin (OP), and osteocalcin (OC)] genes was detected by cellular mRNA levels at day 7 (ROB: proliferating) and 27 (ROB: differentiated) in primary cultures of normal diploid, calvarial-derived rat osteoblasts and at days 2 (ROS: proliferating) and 7 (ROS: post-proliferative) in ROS 17/2.8 osteosarcoma cell cultures. Analysis of Northern blots was quantitated by microdensitometry, and values are expressed as percentage maximal. These profiles are representative of the patterns of gene expression reported for independent cell preparations.

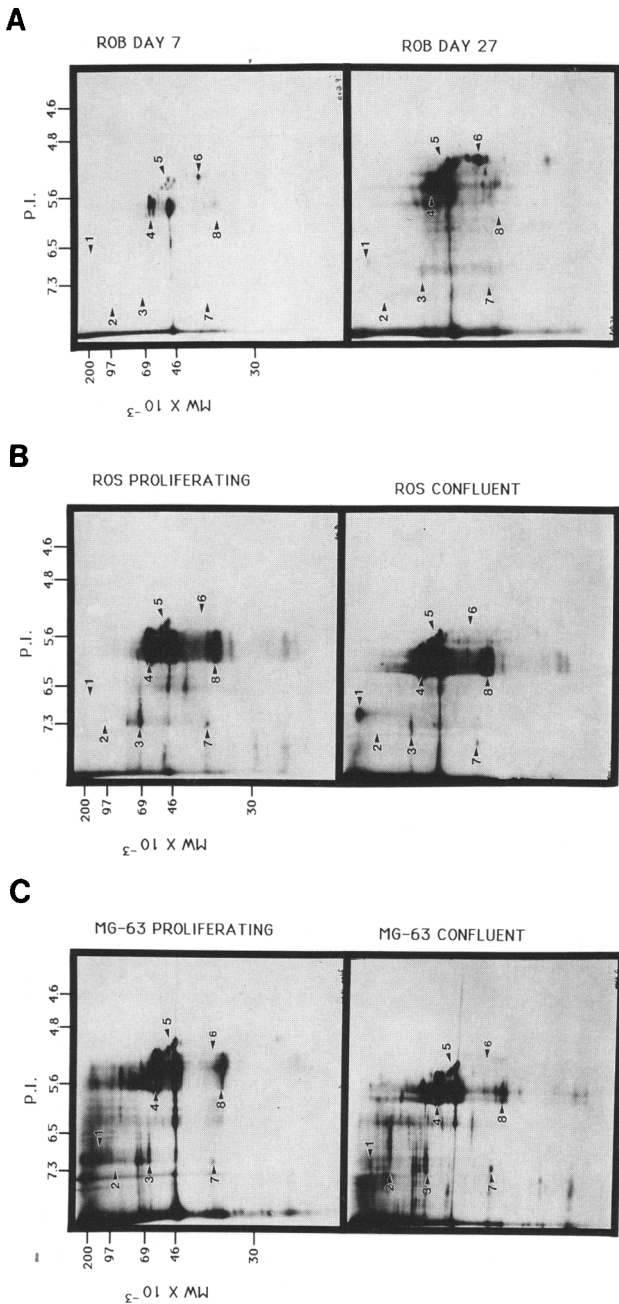


Fig. 2. Comparison of the representation of nuclear matrix proteins in normal diploid osteoblasts (A), ROS 17/2.8 rat osteosarcoma cells (B), and MG-63 human osteosarcoma cells (C). Day 7 (proliferating) and day 27 (postproliferative) normal diploid osteoblasts as well as proliferating (day 2) and day 7 (postproliferative) ROS 17/2.8 or MG-63 osteosarcoma cells were radiolabeled with [35 S]methionine. Nuclear matrix-intermediate filament proteins were isolated and subjected to two-dimensional electrophoretic analysis. Examples of nuclear matrix proteins which are preferentially expressed in differentiated, postproliferative normal diploid osteoblasts that are expressed in both proliferating and postproliferative osteosarcoma cells are designated 4 and 5. Proteins observed only in osteosarcoma cells are designated 1-3, 7, and 8. A protein expressed in normal diploid osteoblasts and absent in osteosarcoma cells is designated 6. The representation of designated nuclear matrix proteins was verified in at least three independent experiments.

matrix parallel the sequential expression of genes during the progressive development of the osteoblast phenotype (38). The composition of the nuclear matrix is constant within each of the three principle periods of osteoblast differentiation but is modified dramatically at the two key transition points: at completion of the proliferation period and at the onset of extracellular matrix mineralization. This relationship between nuclear matrix protein composition and the expression of specific genes is further supported by retention of the characteristic

stage-specific representation of nuclear matrix proteins when the osteoblast developmental sequence is delayed (38).

As indicated in Fig. 2 and schematically illustrated in Fig. 3, comparison of proliferating normal diploid osteoblasts (day 7) with postproliferative osteoblasts (day 27) indicates a developmental progression of nuclear matrix protein alterations that are similar to those we described previously (38). Particularly evident is the increased expression of proteins designated 4 and 5 in postproliferative osteoblasts during the extracellular matrix maturation and mineralization stages of differentiation (Figs. 2A and 3A). Here we demonstrate that in striking contrast, these proteins are expressed at similar and high levels in both proliferating and postproliferative rat (Figs. 2B and 3B) as well as human (Figs. 2C and 3C) osteosarcoma cells.

Tumor-related Nuclear Matrix Proteins Characterize the Osteosarcoma Phenotype. In addition to the coexpression of nuclear matrix proteins in osteosarcoma cells that are represented in a mutually exclusive developmental manner during osteoblast differentiation, other nuclear matrix proteins are unique to normal diploid osteoblasts and osteosarcoma cells. A predominantly represented M_r 40,000 nuclear matrix protein (designated 6) is expressed only in osteoblasts and not in rat (ROS 17/2.8) or human (MG-63) osteosarcoma cell lines (Figs. 2 and 3). The two-dimensional patterns of nuclear matrix proteins in the osteosarcoma cells indicates at least five major proteins, the expression of which is either specific or significantly elevated. These proteins have apparent molecular weights of 35,000-38,000 (designated 7 and 8), 72,000 (designated 3), 95,000 (designated 2), and (designated 1). Expression of the M_r 95,000 and M_r 180,000 proteins is tumor specific and growth dependent, while the proteins designated 3, 7, and 8 are tumor specific and proliferation independent.

Nuclear Matrix Proteins as Markers for Osteosarcoma. We have established that the representation of nuclear matrix proteins characterizes the osteosarcoma phenotype from three standpoints: (a) coexpression of nuclear matrix proteins restricted to the proliferation or postproliferative developmental stages of osteoblast differentiation; (b) the presence of two unique classes of nuclear matrix proteins not represented in normal diploid osteoblasts; and (c) an absence of nuclear matrix proteins found only in normal diploid osteoblasts.

The simultaneous expression of genes in osteosarcoma cells which are developmentally expressed in a sequential and mutually exclusive manner in normal diploid osteoblasts suggests a functional relationship between tumor-related modifications in nuclear matrix proteins and those observed in the expression of cell growth and tissue-specific genes. Such a regulatory role for nuclear matrix proteins is consistent with the association of cell growth and bone-specific transcription factors with the nuclear matrix (24, 39, 40) and growth-related partitioning of oncogene-encoded transcription factors between the nuclear matrix and nonmatrix nuclear fraction in a proliferation-dependent manner (39). Involvement of the nuclear matrix in the processing of primary gene transcripts, and particularly the targeting of mRNAs, may also be important in this context (27).

It must be acknowledged that the transcription regulatory mechanisms by which the nuclear matrix contributes to modifications in the extent to which proliferation and phenotypic genes are expressed in transformed and tumor cells remains to be determined. However, there is a striking representation of nuclear matrix proteins unique to cells, tissues, and developmental stages of differentiation and tissue organization. Together with selective association of regulatory molecules with the nuclear matrix in a growth- and differentiation-specific manner there is a potential for the application of nuclear matrix proteins to tumor diagnosis, assessment of tumor progression, and prognosis of therapies where properties of the transformed state of cells is modified.

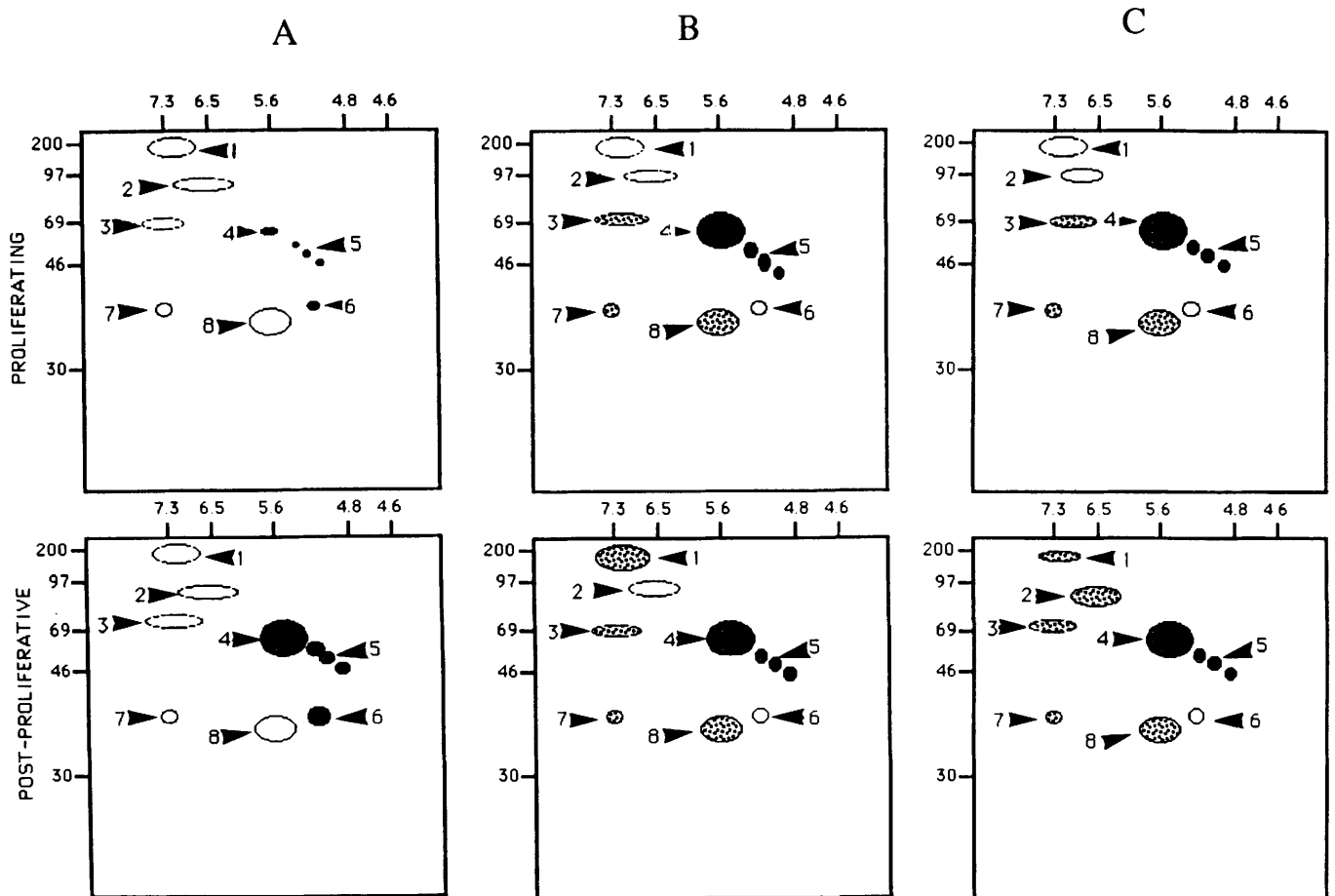


Fig. 3. Schematic representation of nuclear matrix proteins in normal diploid osteoblasts (A), ROS 17/2.8 rat osteosarcoma cells (B), and MG-63 human osteosarcoma cells (C). The nuclear matrix proteins resolved in the two-dimensional gels shown in Fig. 2 are designated as follows: *solid symbols* (designated 4 and 5) indicate the presence of nuclear matrix proteins characteristic of postproliferative normal diploid osteoblasts that are coexpressed in both proliferating and postproliferative osteosarcoma cells or protein 6, which is proliferation independent but only observed in normal diploid cells. The tumor-specific nuclear matrix proteins are designated by *stippled symbols*. *Unfilled symbols* indicate the absence of designated nuclear matrix proteins.

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