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Nuclear Microenvironments in Cancer Series

Chromatin Organization and Nuclear Microenvironments in Cancer Cells

Shihua He, Katherine L. Dunn, Paula S. Espino, Bojan Drobic, Lin Li, Jenny Yu, Jian-Min Sun, Hou Yu Chen, Susan Pritchard, and James R. Davie*

Manitoba Institute of Cell Biology, University of Manitoba, Winnipeg, Manitoba Canada R3E 0V9

Abstract Nuclear morphometric descriptors such as nuclear size, shape, DNA content and chromatin organization are used by pathologists as diagnostic markers for cancer. However, our knowledge of events resulting in changes in nuclear shape and chromatin organization in cancer cells is limited. Nuclear matrix proteins, which include lamins, transcription factors (Sp1) and histone modifying enzymes (histone deacetylases), and histone modifications (histone H3 phosphorylation) have roles in organizing chromatin in the interphase nucleus, regulating gene expression programs and determining nuclear shape. Histone H3 phosphorylation, a downstream target of the Ras-mitogen activated protein kinase pathway, is involved in neoplastic transformation. This article will review genetic and epigenetic events that alter chromatin organization in cancer cells and the role of the nuclear matrix in determining nuclear morphology. J. Cell. Biochem. 104: 2004–2015, 2008.

Key words: nuclear structure; chromatin modifying enzymes; H3 phosphorylation; nuclear matrix

Multistep tumorigenesis is a progression of events resulting from alterations in the processing of the genetic information. These alterations result from stable genetic changes (mutations) in tumor suppressor genes and oncogenes (e.g., *ras*) and potentially reversible epigenetic changes (i.e., modifications in gene function without a change in DNA sequence) [Hake et al., 2004; Vogelstein and Kinzler, 2004; Espino et al., 2005]. DNA methylation and histone modifications are two epigenetic mechanisms that are altered in cancer cells [Esteller, 2007].

The activation or repression of mammalian genes involves chromatin remodeling by histone modifying enzymes and ATP-dependent chromatin remodeling complexes (e.g., SWI/SNF; Fig. 1). Histone acetyltransferases (HATs) and histone deacetylases (HDACs), which catalyze reversible histone acetylation (Ac), are among the best understood chromatin modifying enzymes in terms of multiprotein components, mechanisms of recruitment to regulatory elements of genes and role in transcription. Transcription factors recruit coactivators with HAT activity (e.g., p300/CBP) to regulatory DNA sites, while transcriptional repressors recruit corepressors with HDAC activity [Espino et al., 2005]. In transcriptionally poised and active chromatin regions histone Ac is a dynamic process, with the steady state of acetylated histones being decided by the relative activities of the recruited HAT and HDAC complexes [Davie, 2003a]. Histone kinases [e.g., the H3 kinase, mitogen and stress activated kinase (MSK1)] are recruited to promoters by similar processes. The ATP-dependent chromatin remodeling complexes remodel nucleosomes allowing transcription factors and the transcription initiation factors access to regulatory DNA sequences [Langst and Becker, 2004]. The temporal order by which histone modifying enzymes and ATP-dependent chromatin remodeling complexes are recruited

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*Correspondence to: James R. Davie, Manitoba Institute of Cell Biology, 675 McDermot Avenue, Winnipeg, Manitoba, Canada R3E 0V9. E-mail: davie@cc.umanitoba.ca

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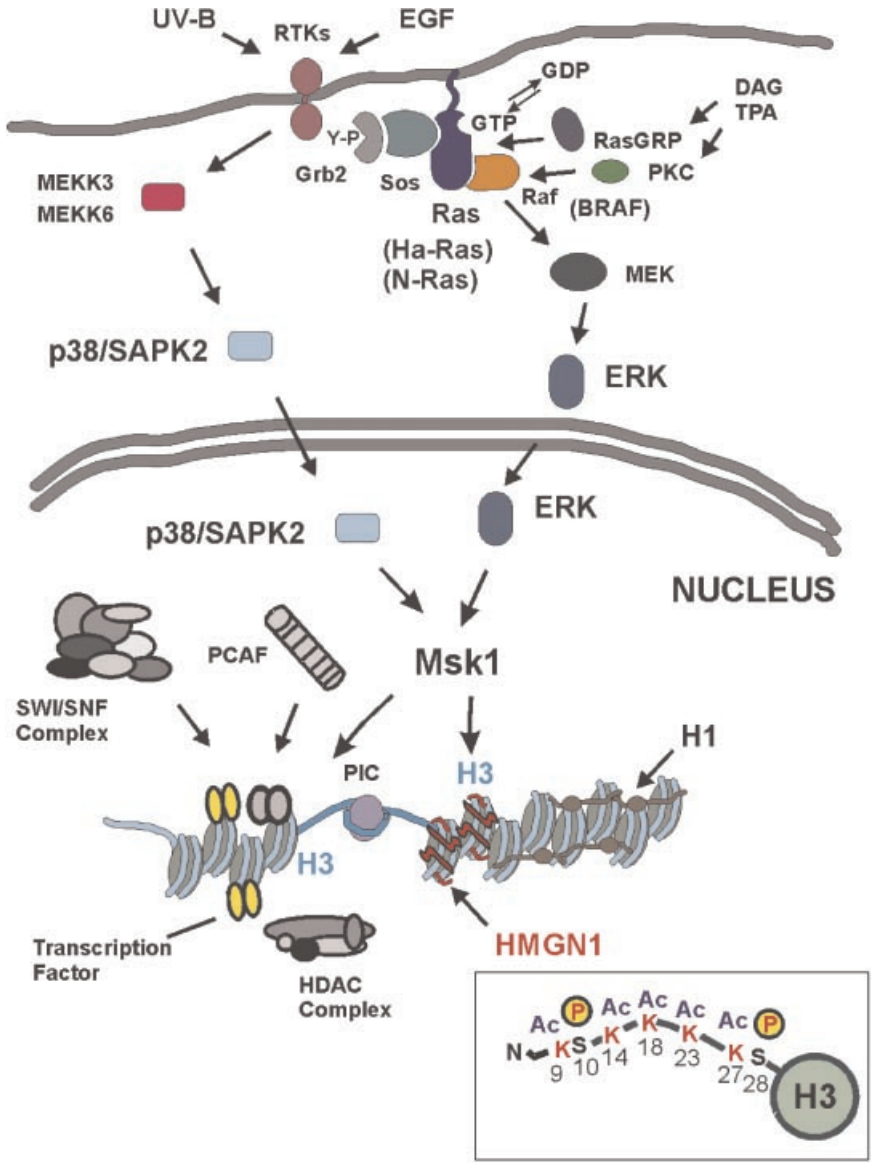

Fig. 1. MAPK signal transduction pathways and the modification of chromatin. The Ras–MAPK pathway is activated by EGF and TPA. RTKs, receptor tyrosine kinases; RasGRP, Ras guanyl nucleotide-releasing protein; DAG, diacylglycerol; PIC, preinitiation complex [Espino et al., 2005]. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

to DNA is promoter context dependent [Lewis and Reinberg, 2003; Martens et al., 2003; Vermeulen et al., 2003].

Phosphorylation of histone H3, a downstream target of several signaling pathways, is an important event in neoplastic transformation [Dong and Bode, 2006]. Growth factors (epidermal growth factor, EGF) and phorbol esters (TPA) activate the Ras–MAPK pathway (Ras–Raf–MEK–ERK), while stressors such as UV irradiation stimulate the p38 MAPK pathway (Fig. 1). Stimulation of the Ras–MAPK pathway results in the activation of a series of kinases and transcription factors and the modification of chromatin proteins [Hazzalin and Mahadevan, 2002]. TPA or EGF stimulation of mouse fibroblasts and human cell lines (HeLa, HEK293, MCF-7) results in the phosphorylation of H3 and its variants at S10 and S28 (but not S28 in MCF-7 cells) and HMGN1 at S6 [Strelkov and Davie, 2002; Soloaga et al., 2003; Lim et al., 2004; Dunn and Davie, 2005; Espino et al., 2006] (Fig. 1). The amplitude and duration of TPA-induced phosphorylation of H3

at S10 and S28 are similar, with the duration varying with cellular background. Treatment of cells with MEK inhibitors before EGF or TPA stimulation blocks these phosphorylation events [Chadee et al., 1999; Lim et al., 2004; Dunn and Davie, 2005].

In TPA-treated serum-starved mouse fibroblasts, newly phosphorylated S10 H3 is distributed as numerous small foci scattered throughout the interphase nuclei; these foci are found outside condensed chromatin regions [Chadee et al., 1999]. We obtained similar results with TPA-stimulated HeLa and MCF-7 cells [Espino et al., 2006]. We proposed that these H3S10p nuclear sites represent the locations of genes [e.g., intermediate early (IE) gene response] that were induced or in a competent state. To test this idea, we incubated cells with 5-fluorouridine to label newly synthesized nuclear RNA and found that many H3S10p sites were positioned next to regions with newly synthesized RNA after TPA stimulation of MCF-7 cells [Espino et al., 2006].

TPA-induced H3S10p and H3S28p are associated with different chromatin regions in mouse fibroblasts [Dunn and Davie, 2005; Dyson et al., 2005]. These results were also reported by Mahadevan's group in a study demonstrating that the H3S10p and H3S28p are not localized on the same H3 tail in situ [Dyson et al., 2005]. We also observed that the H3S10p and H3S28p foci were co-localized with RNA polymerase II, providing evidence that both modifications are associated with transcribed chromatin (unpublished observations). Immunoblot analyses of H3 resolved by size and charge by acetic acid urea PAGE revealed that H3S28p was associated with the more acetylated H3 isoforms in contrast to H3S10p [Dunn and Davie, 2005]. This differential distribution of phosphorylated H3 isoforms was not due to epitope exclusion of antibodies [Dunn and Davie, 2005]. Our data suggest that H3S28p is in chromatin regions with greater steady state levels of acetylated H3 than is H3S10p.

Although there are several kinases that will phosphorylate H3, including RSK2 and tissue transglutaminase 2, which we reported is an H3 kinase, MSK is the mitogen-stimulated H3 kinase [Davie, 2003b; Bode and Dong, 2005]. Unlike RSK2, MSK is activated by Ras–MAPK and p38 stress kinase pathways [Soloaga et al., 2003; Fig. 1]. Studies with MSK1/2 knockout cells show severe reduction of H3S10p and H3S28p in response to TPA. The remaining H3 phosphorylation was due to mitotic phosphorylation by Aurora B of H3 in late G2 phase of the cell cycle [Goto et al., 2002; Soloaga et al., 2003]. TPA-induction of c-*fos* was significantly decreased in the double MSK1/2 knockout mouse [Soloaga et al., 2003].

In vitro MSK will phosphorylate an H3 at S10 and S28; however, in situ the enzyme typically phosphorylates an H3 at S10 or S28 [Lim et al., 2004; Dunn and Davie, 2005; Dyson et al., 2005]. Recent literature indicates that this may be due to a histone code present prior to mitogen stimulation and maintained throughout induction. Studies by Hazzalin and Mahadevan determined that H3 trimethylated at K4 is present in nucleosomes associated with specific regions of c-*fos* and c-*jun* before and during exposure to TPA [Hazzalin and Mahadevan, 2005]. Further, our studies have found a difference in the relative level of Ac present on H3 phosphorylated at S10 and S28 following mitogen stimulation. Tails targeted for phosphorylation at S10 tended to be undergoing steady-state Ac to a lesser extent than those tails targeted for phosphorylation at S28 [Dunn and Davie, 2005]. Other possible mechanisms by which substrate specificity of MSK is altered in situ include but are not limited to: Ac and/or methylation of H3 at specific sites direct MSK1/2 activity to S10 or S28 (i.e., a histone code); promoter context and mechanism of MSK recruitment direct MSK to either S10 or S28; a protein (e.g., HAT interacting with a nearby K) blocks MSK access to S10 or S28; or chromatin structure. Further studies are underway to sort through these various mechanisms.

Others and we applied the chromatin immunoprecipitation (ChIP) assay to directly demonstrate that TPA/EGF-induced H3S10p is associated with promoter and coding regions of IE genes (c-*jun*, c-*fos*, and c-*myc*) in mouse fibroblasts [Chadee et al., 1999; Clayton et al., 2000; Thomson et al., 2001]. Inhibition of MEK activity with PD98059 or UO126 prevented TPA induction of these genes. Further, treating cells with the MSK inhibitor H89 prevented TPA-induced phosphorylation of S10 H3 and attenuated IE gene expression but did not diminish ERK phosphorylation [Thomson et al., 1999; Strelkov and Davie, 2002]. Also,

there was an increase in the steady state of acetylated H3 bound to transcribed chromatin of IE genes c-*jun* and c-*fos* following stimulation of the MAPK pathway [Thomson et al., 2001].

We extended our ChIP assays to analyze the TPA-/estrogen-responsive gene, trefoil factor 1 (TFF1), in human breast cancer MCF-7 cells [Espino et al., 2006]. TFF1 is a small-secreted protein that acts as a proinvasive and angiogenic agent [Prest et al., 2002; Rodrigues et al., 2003a,b]. TPA addition to MCF-7 cells cultured under estrogen-free serum starved conditions results in the phosphorylation of ERKs and S10 H3 and TFF1 gene expression. TPA-, but not estrogen-, induced TFF1 expression was diminished with the MEK inhibitor UO126 and MSK inhibitor H89. The TFF1 promoter has a Sp1 site, an estrogen responsive element and two AP-1 sites with the upstream site being imperfect. Our transient transfection studies demonstrated that the downstream AP-1 is required for TPA-stimulation of the TFF1 promoter. In ChIP assays we found that following 30 min of TPA stimulation, there was an increased association of c-Jun, MSK1, acetylated H3 and H3S10p with the promoter [Espino et al., 2006].

The mechanism(s) by which MSK is recruited to target genes is poorly understood. One mechanism would be that MSK is loaded onto target promoters before induction. However, ChIP assays indicate that MSK1 is loaded onto the IL-6 promoter after cells are stimulated (TNF) [Vermeulen et al., 2003]. MSK1 binds to and phosphorylates p65 subunit of NF-κB. Therefore, it is likely that NF-κB is involved in recruitment of MSK1 to the IL-6 promoter and perhaps other NF-kB responsive genes [Vermeulen et al., 2003]. Treatment of mouse fibroblasts with TNF and PD98059 or H89 prevented the recruitment of MSK1 to the IL-6 promoter. In contrast to IL-6, p65 may recruit MSK to the c-*fos* promoter before induction as p65 is constitutively loaded onto the c-*fos* promoter [Anest et al., 2004]. Our ChIP data with the TFF1 promoter in MCF-7 cells show that MSK1 binding to this promoter increases after TPA [Espino et al., 2006]. Further, we used a formaldehyde cross-linking strategy to show that MSK1 is bound to chromatin before and after TPA stimulation of mouse fibroblasts and MCF-7 cells [Dunn et al., 2005]. These results do not tell us if MSK1 is bound directly or indirectly to DNA as formaldehyde cross-links protein to DNA and protein to protein. We favor the idea that MSK1 is indirectly recruited to a promoter by a transcription factor or co-activator.

Fos and Jun are the prime suspects for recruitment of MSK to the TFF1 promoter. Fos is a substrate for MSK2 (also named RSK-B) in vitro, and MSK2 stimulates the activity of AP-1 driven promoters in transient transfection assays [Pierrat et al., 1998]. Our model is that Fos and/or Jun would recruit MSK1/2 to the TFF1 promoter. MSK1/2 would then be positioned to phosphorylate H3 in nucleosomes placed at and near the promoter. Note that depending on the compaction state of chromatin, which for transcribed chromatin may be at least 40-fold, MSK positioned at the promoter could access nucleosomes at the promoter and those within the coding region. As our studies demonstrate that H3 phosphorylation occurs in the coding region of stimulated genes, there are likely mechanisms by which MSK are recruited to these downstream regions in addition to the promoter [Chadee et al., 1999; Dunn and Davie, 2005].

Steady-state levels of pERKs (not p38), H3S10p/H3S28p and phosphorylated H1 are elevated in Ha-*ras*-transformed mouse fibroblasts (Ciras-3) relative to the parental 10T½ cells [Chadee et al., 1995, 1999, 2002]. Similar results were obtained in other cellular backgrounds with oncogenes impacting on the Ras–MAPK pathway [Chadee et al., 1999]. The increased phosphorylation of H3 and H1 likely contributes to the less condensed chromatin structure of Ciras-3 cells relative to parental cells [Chadee et al., 1995]. The increased level of H3S10p/H3S28p was a consequence of increased MSK1 (and likely MSK2) activity in the Ciras-3 cells [Drobic et al., 2004]. The H3 phosphatase PP1 activity was similar in both cell lines. The cellular distribution of MSK1 and 2 was the same in the Ciras-3 and 10T½ cells, with most MSK1/2 being in the nucleus. Interestingly H89 had a more profound effect on reducing the TPA-induced phosphorylation of S10 H3 and the induction of genes c-*fos*, c-*myc* and urokinase plasminogen activator in Ciras-3 than in parental cells [Strelkov and Davie, 2002]. Possibly, genes responding to a constitutively activated Ras–MAPK and increased MSK activity are more reliant or addicted [Jonkers and Berns, 2004] to this

pathway for expression than those of the parental cell line.

HISTONE MODIFICATIONS AND CANCER DIAGNOSIS

Histone H3 phosphorylation at S10 and S28 is associated with transcriptionally active chromatin and hence is considered as an active mark. Further, Ac of the core histones H2A, H2B, H3, and H4 and dimethylation (diMe) or trimethylation (triMe) of K4 of H3 are active marks, while diMe or triMe of H3 at K9 constitutes a repressive mark [Peterson and Laniel, 2004; Sims and Reinberg, 2006]. Added to the complexity are histone variants and the dynamic reversibility of some modifications [Clayton et al., 2006]. H2A, H2B, and H3 have variants, some expressed at the time of DNA synthesis (H3.1, H2A.1) and others expressed throughout the cell cycle (H2A.Z, H3.3) [Jin et al., 2005].

Altered expression or aberrant targeting of histone modifying enzymes is a common occurrence in cancer cells. Chromosomal translocation events occurring in leukemia result in the genesis of fusion proteins such as AML1-ETO in acute myeloblastic leukemia and PML-RARalpha in acute promyelocytic leukemia. These fusion proteins "highjack" the HDACs enzymes to promoters, resulting in their silencing [Trus et al., 2005; Minucci and Pelicci, 2006]. The HAT p300 appears to be involved in prostate cancer cell proliferation and is a biomarker to predict aggressive features of prostate cancer [Debes et al., 2003]. Interestingly, the increased expression of p300 in prostate cancer cells correlated with changes in nuclear shape. The changes in nuclear shape were thought to be due to increased expression of lamin A/C [Debes et al., 2005].

Changes in the expression levels or mistargeting of histone modifying enzymes may result in alterations in steady state levels of histone modifications which may have predictive power in terms of diagnosis, prognosis and disease correlation [Hake et al., 2004]. Global changes in histone modifications have been used to group patients with low-grade prostate cancer into two groups with differential risks of tumor recurrence. The group with lower risk of tumor recurrence had higher steady levels of H3K18Ac, H3K9Ac, H4K12Ac, and H3K4diMe [Seligson et al., 2005]. A challenge in doing these immunohistochemistry assays is for the histone modification to be preserved, and this is particularly acute for phosphorylation.

NUCLEAR STRUCTURE AND NUCLEAR MATRIX

Pathologists have long used nuclear shape as a diagnostic tool to distinguish a normal cell from a cancer cell [Nickerson et al., 1995; Coffey, 2002]. Nuclear morphometric descriptors included nuclear size, shape, DNA content and chromatin organization. Unfortunately a detailed understanding to explain these changes and how they arise is not available. Nuclear size and shape is determined, in part, by the dynamic nuclear substructure, the nuclear matrix. The nuclear matrix also has a role in chromatin organization, and this nuclear substructure may have a functional role in events leading to genomic instability and changes in DNA content.

The nuclear matrix consists of both nuclear proteins and RNA. The nuclear matrix proteins may be analyzed by isolating the nuclear matrices typically by DNase I digestion and extractions with 0.25 M ammonium salt [Samuel et al., 1997]. Milder methods for isolating nuclear matrices are also available [Jackson and Cook, 1985]. A cisplatin cross-linking protocol to isolate nuclear matrix proteins that are associated with nuclear DNA may also be used [Spencer et al., 2001].

Nuclear matrix proteins (NMPs) represent about 30% of the nuclear protein. This subset of the cellular proteome includes proteins with roles in the organization and function of nuclear DNA. NMPs are involved in establishing chromatin loop domains and in the organization of chromosome territories [Coffey, 2002; Jackson, 2003]. The nuclear matrix has a pivotal role in the processing of the genetic information [Stein et al., 2004]. DNA replication, transcription and DNA repair occur at defined nuclear matrix sites [Dimitrova and Berezney, 2002; Jackson, 2003]. Transcription factors including tumor suppressors (e.g., Rb, p53) and hormone receptors (estrogen receptor) dynamically associate with specific nuclear matrix sites [Davie et al., 1998; Stenoien et al., 2000]. The cancer cell nuclear matrix proteome has proteins involved in the aberrant processing of the genetic information, the disorganization of the genome, and altered nuclear structure. As such, these NMPs are potential biomarkers of the disease.

Human NMPs fall into at least three groups; NMPs expressed in most cell types (e.g., heterogeneous nuclear ribonucleoproteins), cell type specific NMPs, and disease state specific NMPs [Khanuja et al., 1993; Replogle-Schwab et al., 1996; Samuel et al., 1997, 1998]. It is this last group that has been most informative in cancer detection and diagnosis [Hughes and Cohen, 1999; Leman and Getzenberg, 2002; Luftner and Possinger, 2002]. NMPs specific to breast, urothelial, bladder, colon and prostate cancer have been identified [Luftner and Possinger, 2002]. These cancer specific NMPs with biomarker potential may include fusion transcription factors (e.g., ETO-AML1 [Barseguian et al., 2002]) or proteins aberrantly expressed in cancer cells (e.g., an embryonic-specific protein). Apoptosis or conditions like leaky tumor vasculature can cause leakage of cancer type specific NMPs in the bloodstream [Keesee et al., 1996; Luftner and Possinger, 2002]. Thus NMPs have great potential in the detection and diagnosis of cancer and clinical trials are underway to test the specificity and sensitivity of certain NMPs in cancer detection [Luftner and Possinger, 2002]. As NMPs remain in circulation after the death of cancer cells for sufficient time to induce an immune response [Miller et al., 1992], another strategy is to detect antibodies against the disease specific NMP. One success story came from the discovery that NMP22 detected bladder cancer, leading to the genesis of a U.S. Food and Drug Administration-approved in-office test for bladder cancer (NMP22 BladderChek® sold by Matritech, Inc.; http://www.matritech.com/).

Enzymes involved in chromatin remodeling such as SWI/SNF, HAT, and HDACs are associated with the nuclear matrix (Figs. 2 and 3). In an analyses of HDACs associated with MCF-7 breast cancer cells, we found that HDAC1, HDAC2, HDAC3 but not HDAC4 were associated with the nuclear matrix [Sun et al., 2001]. How the HDACs targeted to the nuclear matrix is unknown. Presumably the proteins will have a nuclear matrix-targeting domain as identified for the RUNX transcription factor [Harrington et al., 2002]. F-actin, a component of the nuclear matrix, is involved in the nuclear matrix binding of the HDACs as well as p53 [Okorokov et al., 2002; Andrin and Hendzel, 2004; McDonald et al., 2006]. It will be interesting to determine whether HDACs 1 and 2 bind directly to actin or more likely to an actin binding protein.

Nuclear shape and chromatin appearance are dramatically different in some oncogene-transformed mouse fibroblasts, where these changes in nuclear shape and chromatin organization correlated with the metastatic

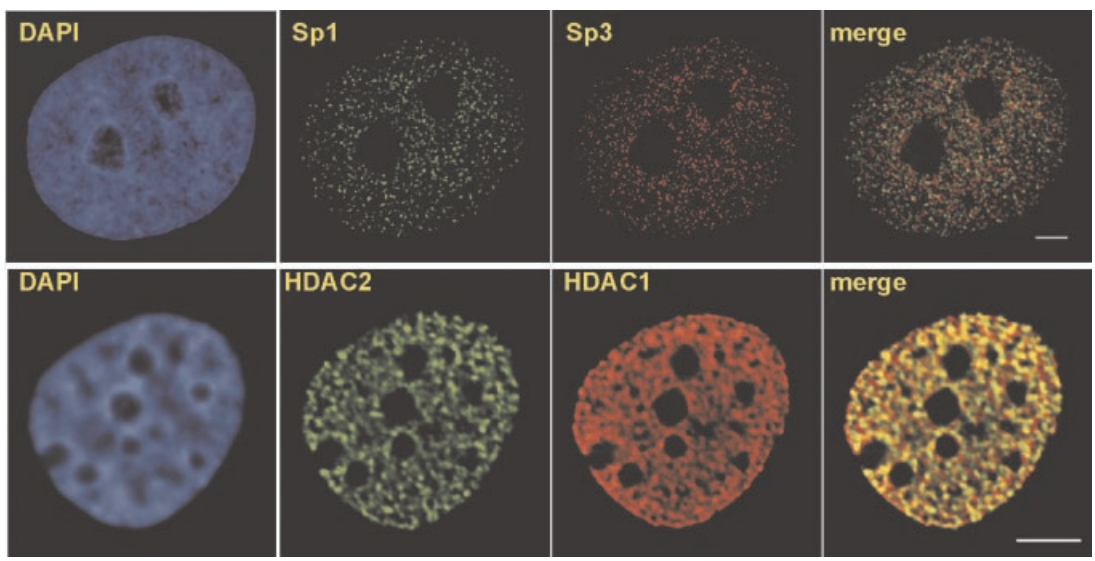

Fig. 2. Intranuclear distribution of Sp1, Sp3, HDAC1 and HDAC2 in MCF-7 cells. MCF-7 cells were grown on coverslips in estrogen complete medium, fixed, and double labeled with anti-Sp1 and anti-Sp3 or anti-HDAC1, and anti-HDAC2 antibodies. DNA was stained by DAPI. Sp1 and Sp3 as well as HDAC1 and HDAC2 distributions were visualized by fluorescence microscopy and image deconvolution as described in He et al. [2005]. Yellow in the merged images signifies colocalization. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

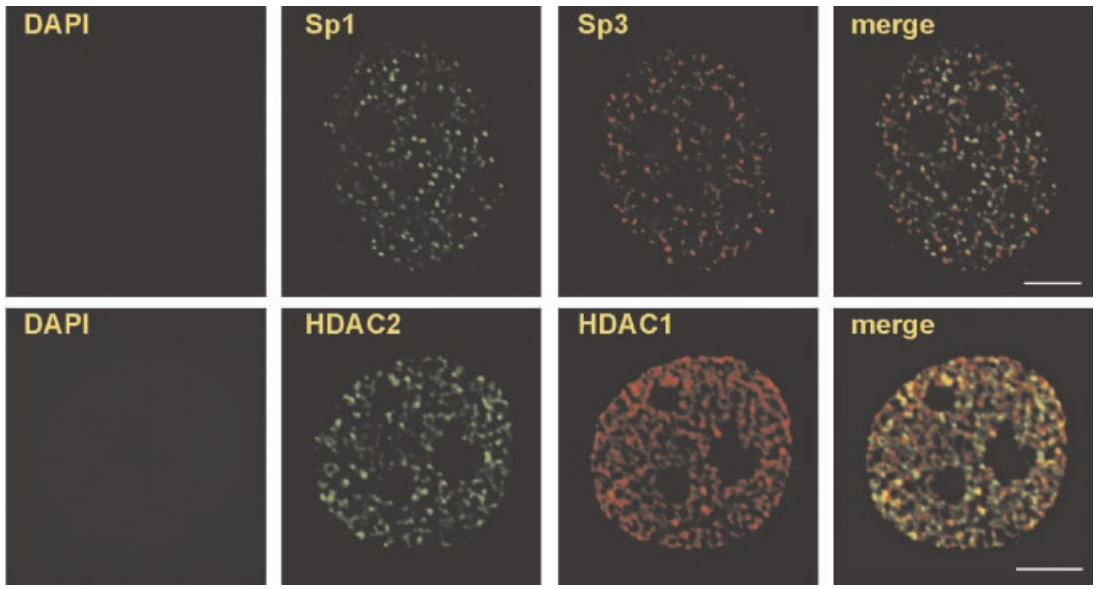

Fig. 3. Nuclear matrix distribution of Sp1, Sp3, HDAC1, and HDAC2. MCF-7 cells were grown on coverslips in estrogen complete medium, and nuclear matrices were prepared as described in He et al. [2005]. The remaining nuclear matrix was double labeled with anti-Sp1 and anti-Sp3 or anti-HDAC1 and anti-HDAC2 antibodies. Most DNA was removed as made evident by the lack of DAPI staining. Sp1 and Sp3 as well as HDAC1 and HDAC2 distributions were visualized by fluorescence microscopy and image deconvolution. Yellow in the merged images signifies colocalization. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

potential of the cell line. Alterations in the levels of phosphorylated H1 and H3 histones in the oncogene-transformed cells did not correlate with the metastatic potential or changes in nuclear shape. But the oncogene-transformed cell lines with high metastatic potential had radically different NMP profiles and paralleled changes in nuclear shape. It is conceivable that the alterations in NMPs have a role in changing nuclear features.

Nuclear matrix proteins that have roles in nuclear shape are lamins A and C, which are localized to the nuclear periphery and also in the interior of the nucleus [Hutchison, 2002; Lammerding et al., 2006; Bridger et al., 2007]. A multitude of human diseases referred to as laminopathies results from a mutation in type A lamins. Fibroblasts from laminopathy patients have an altered nuclear morphology [Burke and Stewart, 2002]. Further lamins A and C contribute to mechanical stiffness of the nuclei and position of chromosomes in the nucleus [Lammerding et al., 2006; Meaburn et al., 2007]. In analyses of nuclear matrix proteins cross-linked to DNA with cisplatin in a cell line panel that reflected the stages of malignant progression in breast cancer, we observed an increase in nuclear DNA contacts with lamins in cells that had become estrogen-independent and anti-estrogen resistant [Spencer et al., 2001]. These results suggest that as estrogen-responsive breast cancer cells progress towards estrogen independence and antiestrogen resistance, there are changes in contacts between nuclear DNA and NMPs occurring that may alter the nuclear positioning of chromosomal territories and gene expression programs.

Our understanding of the RNA component of the nuclear matrix has lagged behind that of the NMPs. The work of Ron Berezney and colleagues has highlighted the role of RNA not only in nuclear matrix structure integrity but also chromosome territories [Ma et al., 1999]. Nuclear RNAs are likely to be involved in the dynamic nuclear matrix architecture. One example is XIST RNA which is associated with the nuclear matrix [Clemson et al., 1996].

SP1 AND SP3 NUCLEAR LOCATION, FOCI AND CANCER

Sp1 was the first mammalian transcription factor to be purified and characterized. Mammalian cells also express another Sp1-like protein, Sp3. Sp1, and Sp3 regulate the transcriptional activity of many genes involved in

a wide range of biological processes, including differentiation, cell cycle progression, and oncogenesis [Li et al., 2004; Sapetschnig et al., 2004; Jinawath et al., 2005].

Sp1 and Sp3 are localized in distinct non-overlapping foci in the nucleus (Fig. 2) and with the nuclear matrix (Fig. 3) [He et al., 2005]. These sub-nuclear foci containing Sp1 or Sp3 are infrequently associated with sites of transcription. This is a common observation with a number of transcription factors in that these regulatory proteins are localized in foci that infrequently overlap with sites of transcription [Grande et al., 1997]. It has been proposed that the transcription factor foci are involved in transcription factor turnover or stabilization [Stenoien et al., 2000]. Alternatively the foci may be storage sites for transcription factors [Grande et al., 1997]. In the latter case, the foci may function to regulate the levels of Sp1/Sp3 available to associate with interacting proteins and/or regulatory elements. Sp1 and Sp3 interact with several proteins, including chromatin-modifying enzymes (e.g., HATs and HDACs) [Li et al., 2004]. During mitosis, we observed that once the nuclear membrane disassembles in prometaphase Sp1 and Sp3 distribute as separate foci into the cell and are displaced from the chromosomes. Sp3 entered the reforming nucleus before Sp1 preceding RNA polymerase II [He and Davie, 2006].

Over-expression of Sp1 and Sp3 may interfere with their location in the nucleus and in chromatin altering chromatin structure and gene expression programming which in turn may result in cancer. Sp1 and Sp3 protein levels are often greater in cancer cells than in normal cells [Lou et al., 2005]. For example, Sp1 levels were greater in breast carcinomas compared to benign breast lesions [Zannetti et al., 2000]; Sp1 was expressed at higher levels in human hepatocellular carcinomas compared to control livers [Lietard et al., 1997]; Sp1 levels were greater in human thyroid tumors than in normal thyroid tissues [Chiefari et al., 2002]; and Sp1 levels were greater in human gastric cancer tissue than in normal adjacent gastric mucosal tissue [Kitadai et al., 1992; Jiang et al., 2004]. Of note, Sp1 expression is a predictor of survival of gastric cancer [Wang et al., 2003]. Acknowledging that Sp1 over-expression had a role in gastric cancer, it was proposed that reducing the level of Sp1 would reduce the metastatic potential of the gastric cancer cells. Indeed, decreasing expression of Sp1 with Sp1 specific siRNA in gastric cancer cells reduced their growth and metastatic potential when injected into the stomach wall of mice [Jiang et al., 2004]. Further, knocking down Sp1 and Sp3 levels to those of normal cells reduced the potential of fibrosarcoma cells to form tumors in mice [Lou et al., 2005].

Sp1 and Sp3 are involved in the regulation of thousands of genes involved in diverse biological processes [Liang et al., 2004; Jinawath et al., 2005]. A recent ChIP on chip study analyzing the distribution of Sp1 binding along human chromosomes 21 and 22 demonstrated a large number of Sp binding sites [Cawley et al., 2004]. Interestingly only about 20% of the Sp sites were those at the 5′ end of protein coding genes, with about 40% of the sites being located at the 3′ end of non-coding RNA genes. Considering that (1) Sp1/Sp3 binding sites are distributed widely in chromosomes, (2) Sp1/Sp3 overexpression is seen in transformed cells, and (3) reduction of Sp1/Sp3 overexpression prevents transformation (invasion), it is possible that overexpression of Sp1/Sp3 leads to changes in gene expression and chromatin structure.

The nuclear matrix associated Sp1/Sp3 foci may have a role in regulating the levels of Sp1/Sp3 circulating in the nucleus and their dynamic binding to regulatory sequences in the genome. Inappropriate sub-nuclear targeting of Sp1 and Sp3 or exceeding the capacity of the foci to retain Sp1 and/or Sp3 may result in the increased association of Sp1 and Sp3 to their binding sites in chromatin. Note that Sp1 can bind to the Sp site in nucleosomal DNA although at a reduced affinity relative to naked DNA. Alterations in either Sp1 or Sp3 targeting to nuclear matrix sites or changes in the concentrations of these factors could result in aberrant remodeling of chromatin leading to dysfunction of the genome, including genomic instability.

GENOMIC INSTABILITY

Genomic instability is one of the hallmarks of a cancer cell [Boland and Goel, 2005]. Studies by Heng et al. [2006] have found that non-clonal chromosomal aberrations rather than clonal chromosomal aberrations are linked to genomic instability. We have analyzed the chromosomes of cancer cells in culture by spectral karyotyping (SKY). Our studies have revealed that breast

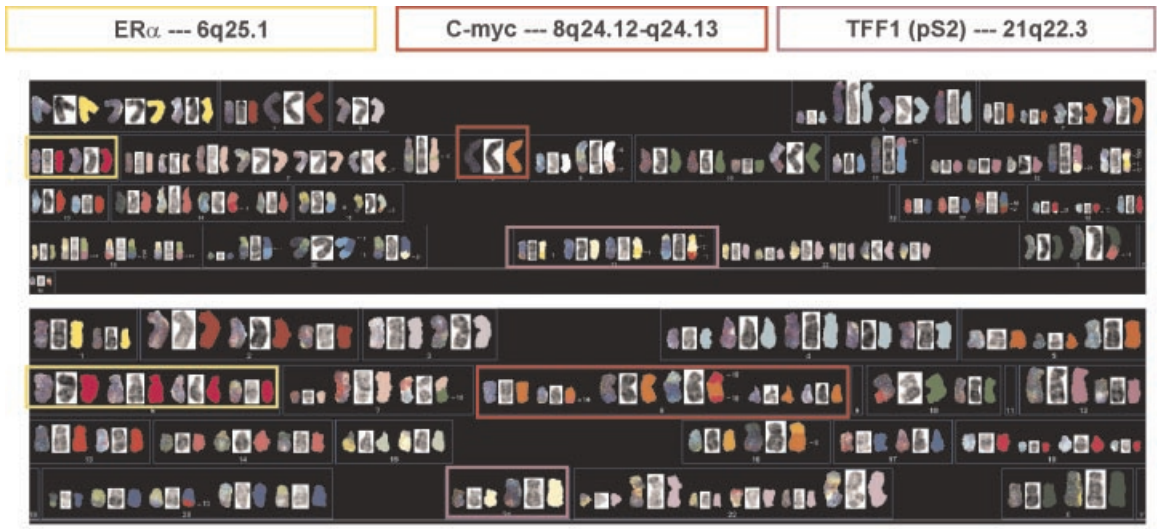

Fig. 4. SKY images of karyotypes of human MCF-7 breast cancer cells. The two karyoypes are substantially different, showing the variation in chromosomes from one MCF-7 cell to the next. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

cancer cells (MCF-7), colorectal cancer cells (HCT-116), and pancreatic cancer cells (Panc-1) are aneuploid. Figure 4 shows two karyotypes of MCF-7 cells. We were surprised to learn that for any given cell line the cell's karyotype was quite variable, providing evidence that each cell in culture has a different chromosome composition. Thus the copy number of any particular chromosome may vary from cell to cell. For example, chromosome 21, which harbors the TFF1 gene (21q22.3), varied from 1 to 5 copies. It is unclear whether all the TFF1 genes in cells with multiple copies of chromosome 21 are estrogen responsive. It is conceivable that pending the nuclear position of the chromosome 21s, the nuclear location of a chromosome 21 may determine whether the TFF1 gene is poised for transcription [Chuang et al., 2006]. Realizing that genomic instability is rampant in these cells, this presents issues with procedures such as the ChIP assay and chromosome genomic hybridization method as both will average the events occurring in the cell population. Clearly, the analyses of transcription factor dynamic loading onto estrogen responsive genes, for example, are being done in a background of genomic instability.

CONCLUSIONS

It is becoming increasingly evident that there is interplay between chromatin modifying enzymes and the nuclear matrix proteins, such as lamins, resulting in an alteration in nuclear shape. It is a rewarding that basic research on HDACs gave rise to an HDAC inhibitor [SAHA also known as vorinostat and Zolinza™ (Merck & Co., Inc.)] being approved by the U.S. Food and Drug Administration for the treatment of cutaneous T-cell lymphoma. It is also exciting that histone modifications may have value in prognosis of cancer types. There are many mysteries in the dynamics of nuclear organization and function that with time will be known. Eventually we will understand the mechanisms involved in altering nuclear shape and explain the changes pathologists have observed for many years to diagnose cancer.

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