Correspondence

Different Chromatin Organization in Benign and Malignant Cells Revealed by Unequal Nuclease Sensitivity between Tumor and Normal Cell Genomes

To the Editor-in-Chief:

We read with great interest the recent article by Maniotis et al,¹ who reported that nuclease-sensitive sites in normal cells were more exposed than those in malignant cells, including normal melanocytes and melanoma cells. The use of nucleases to analyze chromatin organization was initiated by Weintraub and Groudine,² who demonstrated that active genes in normal cells are preferentially digested by DNase I. Subsequently, we³ reported that nuclease accessibility to genomic sites increased following treatment of malignant cells with DNA-modifying agents like bromodeoxyuridine, which concomitantly induces greater cell flattening, increases cell adhesion to substratum,³ and lowers *in vivo* metastasis.⁴ Ours was also the first article that physically isolated hypersensitive DNA and showed unequal hybridization properties between normal and tumor DNA sequences.³ That same year, Puck et al⁵ demonstrated that the morphological reversion of malignant Chinese hamster ovary cells to a normal phenotype by exposure to cyclic AMP derivatives was accompanied by differences in the exposure of DNase I-sensitive sites. However, even after such treatment, malignant Chinese hamster ovary cells remain aneuploid and immortalized, characteristics shared by most malignant cells.⁶

The first article to unambiguously discover that nuclease-sensitive sites in normal cells were more exposed than those in strictly matched malignant cells was published by our group in 1991.⁷ Our novel study to define greater genomic susceptibility in malignancy took special care to compare normal nontumorigenic melanocytes with a diploid chromosome number and the corresponding syngeneic B16 melanoma tumor cells.⁸ Because primary melanocytes derived from fetal or adult skin are not immortalized and do not propagate naturally in culture, 12-O-tetradecanoylphorbol 13-acetate (TPA)⁹ was used in our study⁷ to promote proliferation of primary melanocytes. Hence, under comparable cell cycling conditions, we demonstrated greater genomic susceptibility in melanocytes than melanoma tumor cells. Moreover, TPA-treated melanocytes like those used in our study remain diploid and nontumorigenic, as demonstrated by others.⁸

In contrast, Maniotis et al¹ compared normal melanocytes with malignant melanoma using UM54 normal uveal melanocytes, which may not be syngeneic or matched with OCM1a uveal melanoma cells. In their effort to generalize their findings to several breast tissues, Maniotis et al¹ also compared MCF-10 A normal breast cells with tumorigenic MDA-MB-231 breast carcinoma cells. However, the estrogen receptor-negative status of MCF-10 A normal breast cells is unlike that of strictly normal breast epithelial cells,¹⁰ and its comparison with tumorigenic estrogen receptor-negative, p53-dysfunctional MDA-MB-231 breast carcinoma cells may not be an adequate match.

In their report, Maniotis et al¹ also mentioned "a fundamental difference in the sensitivity of chromatin-associated proteinase K-sensitive proteins between normal and highly invasive cells." We previously demonstrated this when specific ATATAT-rich DNA-binding proteins were implicated in controlling accessibility to DNA in carcinoma chromatin.^{7,11}

For unknown reasons, Maniotis et al¹ omitted citing our precedent work, the first on genomic hypersensitivity in malignancy.^{3,7,11} Therefore, we wish to bring to their attention and that of the readers of *The American Journal of Pathology* that 1) diminished accessibility to chromatin with malignancy was previously demonstrated in syngeneic melanoma versus matched melanocytes,⁷ 2) greater cell adhesion to a matrix was previously shown to increase genomic susceptibility in melanoma,³ and 3) nuclear matrix DNA-binding proteins and DNA precursors like bromodeoxyuridine play a role in controlling accessibility to chromatin.^{3,11} Finally, for accurate assessment of the differences in chromatin accessibility, it remains important that samples are carefully matched between tumor and normal cell populations.

Manuel Rieber
Mary Strasberg-Rieber

Instituto Venezolano de Investigaciones Científicas
Caracas, Venezuela

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Authors' Reply:

In retrospect, had we been aware of the two articles by the Riebers^{1,2} published in *Biochemical and Biophysical Research Communications*, we would have gladly cited them because their work somewhat underscores the species generality of one of our basic findings. However, there seems to be a general lack of awareness of the Riebers' work: of the eight citations made to these articles,^{1,2} only one citation³ originated outside of the Riebers' own laboratory (ISI Web of Knowledge: Web of Science, <http://www.isiwebofknowledge.com/>, accessed November 13, 2006).

To expand further, our findings and those reported by the Riebers are significantly different. In *The American Journal of Pathology*,⁴ we reported that 1) chromatin sensitivity to digestion differs between nonmalignant and malignant human cells and tissues of different origins, 2) increasing sequestration is associated with increasing malignant behavior, 3) chromatin sequestration may depend on nuclear matrix proteins rich in disulfide bonds, 4) extracellular matrix (ECM) composition influences chromatin structure regardless of the degree of malignancy, and 5) ECM-driven chromatin sequestration may be mediated through cytoskeletal components (mechanogenomic signaling⁵).

The Riebers' results differ substantially from our own findings. When the Riebers⁶ applied 12-O-tetra decanoyl phorbol-13 acetate (TPA) to normal mouse melanocytes but not to syngeneic mouse melanoma cells, they may

have overlooked the influence of TPA on actin and microtubule organization^{7,8}: disruption of the cytoskeleton changes the susceptibility of chromatin to digestion.⁴ Furthermore, the Riebers flattened mouse cells after exposure to bromodeoxyuridine (BrdU),⁹ but BrdU may interfere with nuclear matrix DNA-binding and membrane proteins as well as with the cytoskeleton, thereby influencing chromatin digestion. Moreover, the Riebers did not study the relationship of BrdU-treated cells to ECM proteins, a major focus of our investigations. Finally, the Riebers' digestion of chromatin by DNase after cross-linking nuclear matrix proteins associated with AT regions² differs from our use of β -mercaptoethanol or dithiothreitol to reverse chromatin sensitivity to AluI, revealing the importance of disulfide-rich proteins to chromatin exposure.⁴

Although the Riebers' imply that our use of nonsyngeneic cells somehow weakens or invalidates our findings, we introduced our article by stating that we wanted to study "cells of different origins"⁴ because we intended to apply our observations to translational diagnostic and therapeutic settings. It is difficult, if not impossible, to achieve translationally relevant findings using human syngeneic cells without the use of chemicals, viruses, or transgenes. We did not use mouse cells as proof of principle because the distribution of Alu sequences is so different between the mouse and human genomes.¹⁰

Finally, we found that differences in exposure to AluI, DNase I, or MspI were independent of ploidy and cell cycle but were dependent on the composition of the ECM microenvironment, with laminin exerting the most influence.⁴ (It should be noted that there are significant differences between mouse and human laminin.¹¹) Thus, our "syngeneically controlled" experiments with different ECM molecules but with the same cells beautifully demonstrate one of the important conclusions of our article, that the cell's microenvironment profoundly affects the response and organization of chromatin to digestion by these enzymes. We believe these are important and novel findings that in no way overlap with what the Riebers have shown.

Andrew J. Maniotis
 Robert Folberg

University of Illinois at Chicago
 Chicago, Illinois

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