

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

Referentes

1. Maniotis AJ, Valyi-Nagy K, Karavitis J, Moses J, Boddipali V, Wang Y, Nunez R, Setty S, Arbieva Z, Bissell MJ, Folberg R: Chromatin organization measured by AluI restriction enzyme changes with malignancy and is regulated by the extracellular matrix and the cytoskeleton. *Am J Pathol* 2005, 166:1187–1203
2. Weintraub H, Groudine M: Chromosomal subunits in active genes have an altered conformation. *Science* 1976, 193:848–856
3. Rieber M, Strasberg Rieber M: Tumor hypersensitive DNA is enriched in c-myc sequences and reacts differentially with normal and malignant genomic DNA. *Biochem Biophys Res Commun* 1990, 169:352–359
4. Rieber M, Castillo MA: Unequal forms of 140–11-kD glycoproteins in B16 melanoma cells with differing detachment properties and metastatic behaviours: influence of bromodeoxyuridine. *Int J Cancer* 1984, 33:765–770
5. Puck TT, Krystosek A, Chan DC: Genome regulation in mammalian cells. *Somat Cell Mol Genet* 1990, 16:257–265
6. Barranco SC, Shilkun K, Nichols S, Boerwinkle WR, Adams EG, Bhuyan BK: Changes in DNA distributions and ploidy of CHO cells as a function of time in culture. *In Vitro* 1981, 17:730–734
7. Rieber M, Rieber M: Differential genomic susceptibility in malignancy correlates with changes in ATATAT DNA-binding proteins. *Biochem Biophys Res Commun* 1991, 178:1036–1042
8. Bennett DC, Cooper PJ, Hart IR: A line of non-tumorigenic mouse melanocytes, syngeneic with the B16 melanoma and requiring a tumour promoter for growth. *Int J Cancer* 1987, 39:414–418
9. Eisinger M, Marko O, Ogata S, Old LJ: Growth regulation of human melanocytes: mitogenic factors in extracts of melanoma, astrocytoma, and fibroblast cell lines. *Science* 1985, 229:984–986
10. Clarke RD, Howell A, Potten CS, Anderson E: Dissociation between steroid receptor expression and cell proliferation in the human breast. *Cancer Res* 1997, 57:4987–4991
11. Rieber MS, Rieber M: Accessibility to DNA in carcinoma chromatin is promoted by nanomolar okadaic acid: effect on AT-rich DNA binding proteins. *Cancer Res* 1992, 52:6397–6399