The Biology Behind

Nuclear Matrix Proteins as Proteomic Markers of Preneoplastic and Cancer Lesions

Commentary re: G. Brunagel *et al.*, Nuclear Matrix Protein Alterations Associated with Colon Cancer Metastasis to the Liver. Clin. Cancer Res., *8:* 3039–3045, 2002.

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Nuclear Matrix Proteins in Early Cancer Diagnosis

Only a pathologist can diagnose a cancer. This is accomplished by inspecting the visual features of the cancer cells, which include the marked variations in cellular structure termed pleomorphism. Disorganization and pleomorphism are a hallmark of cancer diagnosis and include highly variable structural changes in cell shapes, nuclear and nucleolar shapes, chromatin patterns, and chromosome markers and karyotypes (1, 2). Understanding this pathobiology is of great importance because structural changes in cancer cells are a common denominator, occurring both *in vivo* and in cultured cells as morphological transformation. Furthermore, pleomorphism occurs in all types of animal and human tumors, and this process is initiated early in the preneoplastic stages (2–5). What proteins and biological process might produce these aberrations in tumor cell structure, and how might they be monitored by proteomics? An extraction process reveals the underlying scaffold systems of the cell. The scaffold is apparent if you first extract the total cellular lipids, followed by extracting the soluble proteins by sequentially increasing the salt concentration and including treatment with DNase and RNase (6–8). The residual insoluble protein skeleton that remains determines the overall shape of the cell and many of its internal structures, such as the nucleus. These types of scaffolds are present even in the individual RBCs without a nucleus and increase in complexity in eukaryotic cells with nuclei, all of the way up to forming an overall tissue-scaffolding network. These scaffolds have often been termed skeletons, but they are, in fact, dynamic structures that involve the equilibrium of the polymerization and depolymerization of many structural proteins that form both tension and compression elements such as actin, intermediate keratins, tubulin, and residual components of the nuclear envelope, such as lamins and the insoluble extracellular matrix, and basement membrane components, such as collagen. After the sequential extractions of the bulk of the lipids and soluble biochemical polymers (proteins and nucleic acids), the remaining residual scaffold represents an interconnecting network extending from the extracellular matrix through the internal cytoskeleton to the central nuclear matrix (8). This network system has been termed the tissue matrix (8). After extraction, the overall structures of both the tissue and the cell are still maintained in both normal and cancer cells by this tissue matrix structure. These dynamic structures are believed to form compartments and vectorial transport systems for cellular motor systems to provide directed intracellular transport.

The importance of these compartments and scaffolding systems in the nucleus is that they appear to provide spatial order and DNA domains for individual chromosome domains within the interphase nucleus. They also form fixed loci in the nucleus for individual sites of DNA replication, RNA transcription, and message RNA splicing (9–15). As such, the nucleus appears to be organized differently in different cell types. It is known that an oncogene or suppressor gene can function differently in different cell types, based upon its cellular context. For example, inherited DNA lesions that appear in all cells can cause a high penetrance of colon cancer but do not produce increased rates of prostate cancer. Similarly, inherited prostate cancer genes do not cause colon cancer. Much of this cell context for transformation may be determined by higher order DNA organization extending from chromatin modifications up to higher order loop domains, with each of 50,000 loops in a cell being ~60 kb in DNA length (14). These DNA loop domains are conserved in nature from bacteria to mammalian interphase nuclei, and loops are present in the chromosomes as well as in the sperm (16). It is believed that this organization of loop domains also organizes many of the functional nuclear loci, such as the replisome, transcriptisome, and splicesome (10, 14).

Because the overall DNA sequence remains essentially the same in all somatic cells, it would be anticipated that epigenetic changes in nucleic acids and proteins might account for major aspects of tissue specificity. Indeed, some of the earliest proteomics were applied to the nucleus, and it became clear that one could identify specific tissue and cell types by their proteomic patterns of nuclear matrix proteins (2, 7, 17). These early analyses were first applied to cells in culture, and each cell type could be identified by its proteomic patterns (7). Proteomic changes in the nuclear matrix were expanded to tissue samples, and not only normal but pathological samples could be clearly identified by differences in the nuclear matrix protein patterns. One of the early studies was carried out on rat tissue and was extended to human samples (2, 17). In radical prostatectomy samples, normal benign prostatic hyperplasia and prostate cancer tissue was removed from adjacent areas from the same patient sample, and it became possible to identify the type of pathological lesion through the proteomic pattern of the nuclear matrix (17). Pioneering work in this field has been carried out by Robert Getzenberg, Kenneth J. Pienta and coworkers (2, 11, 17–21), Alan W. Partin (17), and in Sheldon Pennman's laboratory (7) at Massachusetts Institute of Technology. Robert Getzenberg and coworkers extended these types of proteomic studies of nuclear matrix proteins to renal (18) and bladder (19,

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21) cancer and have made the observation that normal bladder tissue would have already undergone some specific changes in its nuclear matrix profile if it was removed adjacent to bladder cancer in comparison with normal bladder tissue taken from patients who did not have cancer. Previously, it had been suspected that there was a field effect associated with cancer in which some of the earliest preneoplastic changes might be detected in normal-appearing adjacent tissues. If this proves to be the case, then these comparative proteomic studies of nuclear matrix proteins could be of paramount importance in early-detection scenarios. Some of these nuclear proteins might also be detected in the urine and blood, and indeed, several groups are studying such a possibility (19, 21). An accompanying paper in this issue by Robert Getzenberg reports changes in nuclear matrix proteins in colon cancer that appear to be specific and associated with liver metastasis. Once again, a field effect of these proteins appears in adjacent normal liver tissues, as was observed earlier in bladder cancer. These nuclear matrix proteins are specific for the type of tumor.

At present, there is much controversy about the biological role and exact nature of the nuclear matrix and its components. There is little doubt that the residual nucleus, after the extractions, is an operational entity, and because the nuclear matrix is different in every cell type, it could be expected to be biologically variable. Nevertheless, the classic work of Berezney and Coffey (15) on DNA replication and the nuclear matrix and the studies of Vogelstein *et al.* (14) on the organization of loop structures and their role in replicon function point to the importance of the nuclear matrix elements in organizing loci within the nucleus. Certainly the nuclear matrix structure is dynamic and will change throughout the cell cycle, and it is my opinion that it is much like a dynamic sponge with open compartments for free diffusion in the nucleoplasm, and that many of the diffusing polymers can interact and polymerize to form these larger, less soluble matrix components, which define chromosomal domains and compartments within the nucleus. Most of the nucleus is open space with chromatin occupying only 35% of the volume, and the matrix, 10%. Certainly, new concepts in self-organization, complexity, and chaos (23) may be involved in understanding how this dynamic process occurs within the nucleus and between cells in their organization into tissues. The old ideas that the nucleus is just a membrane enclosing a bag with spaghetti strands of DNA and chromatin stuffed inside is not the best way to describe the complex compartments and dynamic organization of the nucleus. The vast amount of the DNA should never become entangled and has to be cleanly separated after replication. Many surprises are in store in the future as this dynamic nuclear structure undergoes further refinement and clarification. A host of different biological and polymerizing interactions could make it a key element in organizing nuclear foci for juxtapositioning a large molecular ensemble to perform the molecular machinery of replication, transcription, chromosome dynamics, and cell division, as well as nuclear changes in cell apoptosis. How these structures and organization are perturbed in cancer by the change in structure is an important research question. We have much to learn, but in the meantime, these proteomic marker studies of nuclear proteins and their relation to preneoplastic lesions may become a practical application and could become one of the leading uses of proteomics in the field of cancer studies.

Nuclear Matrix Proteins as Prognostic Factors in Therapy

Nuclear grade has always been a predictor of the aggressiveness of many solid tumors. The higher the grade of nuclear disorganization and perturbed chromatin structure, the more difficult it has been to control the cancer by therapeutics. This is often accompanied by a genetic instability that has produced a background of tumor cell heterogeneity and biological diversity in which clones of cells have potentially different properties and functional differences. This has permitted resistant clones to be selected for continued growth after the initial therapy, and as such, this presents the tumor with an evolutionary-like process for survival. In biological evolution, there is not only mutation but also rearrangements of the chromosomes. It has been rather striking that human DNA and primates have >98% of the same DNA sequence. The expressed genes, however, only represent 5% of the total genome DNA. The remaining 95% of the chromosomal DNA is in the form of repetitive sequences and other forms that are not expressed and, therefore, do not appear for analysis on expression microarrays. Nevertheless, rearrangement or changes in this untranslated DNA could have profound functions on the process of chromosomal organization, cellular function, and survival process. Recently increased attention is turning toward chromosomal instability as a marked feature of cancer, although this had been clearly recognized since the early works of Theodore Boveri in Germany in 1902 (24). These structural and genetic instabilities are now believed by some to be the driving force in developing tumor cell heterogeneity that provide the tumor a variety of subclones for selection for resistance to many forms of therapy. Although there are many specific types of genetic changes associated with cancers, chromosome instability is a common genetic feature of solid tumors (9). These chromosome instabilities can be expressed in many forms including ploidy instability, variation in copy number of individual chromosomes, and marked chromosomal structural instability. These latter changes include marked chromosomal rearrangements that are common in many solid tumor markers. Structural changes may simply rearrange genetic material, such as inversion or balanced reciprocal translocations. More severe changes may result in net gain or loss of genetic material, such as in duplications, deletions, or unbalanced nonreciprocal translocations. The balanced translocations, in which translation swaps material reciprocally between two chromosomes, are very common in the leukemias and lymphomas that can be cured by chemotherapy. In contrast, the unbalanced rearrangements of solid tumors are most often associated with more disorganization that are all most difficult to cure by therapy. The extent of these unbalanced chromosomal rearrangements correlates positively with the level of expression of the nuclear matrix high-mobility-group protein HMGI when tested in human prostate cancer cell lines (9). HMGI, topoisomerase II, and AT-rich sequences have been reported to be located at the base of DNA loop domains in both the nucleus and chromosome and are juxtapositions for chromosomal rearrangements. Transfection and expression of the full-length *HMGI* gene into the LNCap

cell line, which is characterized only by balanced translocations, induces the presence and heterogeneity of unbalanced (nonreciprocal) chromosomal rearrangements but does not increase the balanced rearrangements. How these chromosomal rearrangements are organized and directed by the nuclear matrix proteins such as HMGI can be a complex process, but it certainly seems to be an important area of research (9).

In summary, this is just one case of the potential importance of nuclear structural proteins in the nuclear matrix in understanding chemotherapeutic responses and might provide insight into how some tumors are more easily cured, whereas others are more resistant to therapeutic control. If this does prove to be related to different types of aberrations in chromosomal structures that can be traced to the organization of the DNA on the nuclear matrix, then it would indeed be a new frontier for chemotherapeutic resistance. These types of consideration may soon close the gap in understanding pathology, nuclear structure, chromosome abnormalities, tumor cell heterogeneity, and abnormalities in DNA replication—they could be related by DNA organization on the nuclear matrix. Only research will tell, but it appears to start making sense—form and function—and genetics.

References

1. Pienta, K., Partin, A., and Coffey, D. Cancer as a disease of DNA organization and dynamic cell structure. Cancer Res., *49:* 2525–2532, 1989.

2. Getzenberg, R., Pienta, K., Huang, E., and Coffey, D. Identification of nuclear matrix proteins in the cancer and normal rat prostate. Cancer Res., *51:* 6514–6520, 1991.

3. Boyd, J., Pienta, K., Getzenberg, R., Coffey, D., and Barrett, J. Preneoplastic alterations in nuclear morphology that accompany loss of tumor suppressor phenotype. J. Natl. Cancer Inst., *83:* 862–866, 1991.

4. Partin, A., Steinberg, G., Pitcock, R., Wu, L., Piantadosi, S., Coffey, D., and Epstein, J. Use of nuclear morphometry, Gleason histologic scoring, clinical stage, and age to predict disease-free survival among patients with prostate cancer. Cancer (Phila.), *70:* 162–168, 1992.

5. Mohler, J., Partin, A., Epstein, J., Becker, R., Mikel, U., Sesterhenn, I., Mostofi, F., Gleason, D., Sharief, Y., and Coffey, D. Prediction of prognosis in untreated stage A2 prostatic carcinoma. Cancer (Phila.), *69:* 511–519, 1992.

6. Berezney, R., and Coffey, D. Nuclear Matrix. Isolation and characterization of a framework structure from rat liver nuclei. J. Cell Biol., *73:* 616–637, 1977.

7. Fey, E., and Penman, S. Nuclear matrix protein reflects cell type of origin in cultured human cells. Proc. Natl. Acad of Science USA, *85:* 121–125, 1988.

8. Getzenberg, R., Pienta, K., and Coffey, D. The tissue matrix: cell dynamics and hormone action. Endocr. Rev., *11:* 399–417, 1990.

9. Takaha, N., Hawkins A., Griffin, C., Isaacs, W., and Coffey, D. High mobility group protein I(Y): a candidate architectural protein for chromosomal rearrangements in prostate cancer cells. Cancer Res., *62:* 647–651, 2002.

10. Razin, S. Chromosomal DNA loop constitutes basic unit of the eukaryotic genome organization to evolution. Crit. Rev. Eucaryote Gene Expression, *9:* 279–283, 1999.

11. Pienta, K., and Coffey, D. Nuclear-cytoskeletal interactions: evidence for physical connections between the nucleus and cell periphery and their alteration by transformation. J. Cell. Biochem., *49:* 357–365, 1992.

12. Pienta, K., Getzenberg, R., and Coffey, D. Cell structure and DNA organization. Crit. Rev. Eukaryotic Gene Expression, *1:* 355–385, 1991.

13. Misteli, T., and Spector, D. The cellular organization of gene expression. Curr. Opin. Cell Biol., *10:* 322–332, 1998.

14. Vogelstein, B., Pardoll, D., and Coffey, D. Supercoiled loops and eucaryotic DNA replication. Cell, *22:* 79–85, 1980.

15. Berezney, R., and Coffey, D. Nuclear protein matrix: association with newly synthesized DNA. Science (Wash. DC), *189:* 291–293, 1975.

16. Ward, W., Partin, A., and Coffey, D. DNA Loop domains in mammalian spermatozoa. Chromosoma, *98:* 153–159, 1989.

17. Partin, A., Getzenberg, R., Carmichael, M., Vindivich, D., Yoo, J., Epstein, J., and Coffey, D. Nuclear matrix protein patterns in human benign prostatic hyperplasia and prostate cancer. Cancer Res., *53:* 744–746, 1993.

18. Koney, B., Nangia, A., Nguyen, T., Veitmeier, B., Dhir, R., Acierno, J., Becich, M., Hrebinko, R., and Getzenberg, R. Identification of nuclear matrix protein alterations associated with renal cell carcinoma. J. Urol., *159:* 1359–1363, 1998.

19. Getzenberg, R., Konety, B., Oeler, T., Quigley, M., Hakam, A., Becich, M., and Bahnson, R. Bladder cancer-associated nuclear matrix proteins. Cancer Res., *56:* 1690–1694, 1996.

20. Brunagel, G., Vietmeier, B., Bauer, A., Schoen, R., and Getzenberg, R. Identification of nuclear matrix protein alterations associated with human colon cancer. Cancer Res., *62:* 2437–2442, 2002.

21. Konety, B. R., Nguyen, T. T., Dhir, R., Day, R. S., Becich, M. J., Stadler, W. M., and Getzenberg, R. H. Detection of bladder cancer using a novel nuclear matrix protein. Clin. Cancer Res., *6:* 2618–2625, 2000.

22. Miller, T., Beausang, L., Winchell, L., and Lidgard, G. Detection of nuclear matrix proteins in serum from cancer patients. Cancer Res., *52:* 422–427, 1992.

23. Coffey, D. Self-organization, complexity and chaos: the new biology for medicine. Nat. Med., *4:* 882–885, 1998.

24. Burdette, W. J. The significance of mutation in relation to origins of tumors. A review. Cancer Res., ***15:*** 201–226, 1955.

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