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Nuclear Microenvironments in Cancer Series

The Cancer Nuclear Microenvironment: Interface between Light Microscopic Cytology and Molecular Phenotype

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Abstract A definitive diagnosis of cancer may be rendered by microscopic assessment of only a few cells in an appropriate clinical setting due to the distinctive nuclear structure of most cancer cells in comparison to nuclei of normal human cells. The molecular architecture of non-neoplastic human nuclei—of the nuclear matrix and of matrix-associated proteins and nucleic acids—is being characterized in exquisite molecular detail. What is missing is the application of the findings and tools of molecular biology to understanding the cytological structure of cancer nuclei. This article delves into the basis of nuclear structure at different levels of resolution—light microscopic, electron microscopic, and molecular. J. Cell. Biochem. 104: 1994–2003, 2008. © 2007 Wiley-Liss, Inc.

Key words: nucleus; matrix; cancer; cytology; chromatin

Genetic information packaged in the nuclei of eukaryotic cells generates molecular signals that direct and restrict structural and functional features of the cells in the tissues in which they reside. Although all nuclei of parenchymal cells contain the complete genome of each individual of a species, only a minority of the genes in each cell are expressed. Critical to understanding biologic and disease phenomena is characterization of the mechanisms that regulate expression of relevant genes. One perspective to begin to approach this goal is based on nuclear structure. At the light microscopic level of spatial resolution, the nuclei of different categories of cells have distinctive and characteristic microscopic phenotypes [Demay, 1999]. These cytological differences are sufficiently distinctive and specific for some cells that they provide the basis for classification of these cells. Furthermore, the cytological features of most cancer cells are so distinctive that a specific diagnosis of cancer can be readily made. This paper discusses the relevance of using nuclear structure as the basis for understanding biological and disease phenomena.

NORMAL CELLS WITH DISTINCTIVE NUCLEAR STRUCTURES

A number of cell types have stereotypic nuclear features. Both germ cells and replicating parenchymal cells exhibit a characteristic spectrum of changes in nuclear structure. These series of changes, visible by light microscopy, provide the basis for classifying cells into stages of meiosis and mitosis. The features are based on sequential stages of rearrangement of chromatin and of chromatin-associated proteins. Although providing a basis for delving into the molecular mechanisms of meiosis, these distinctive cytologic appearances of nuclei in human parenchymal cells have little correlation with disease, with the exception of the rare germ tumor of the testes titled spermatocytic seminoma, which has a distinctive pattern of clustering of heterochromatin termed "spiremal." However, in this paper we will not discuss nuclear features of cell replication but will focus on the architecture of interphase nuclei of human somatic cells—normal and neoplastic.

Normal parenchymal cells have relatively similar nuclear features. Subtyping of most

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cells is based on cytoplasmic features of differentiation and on the context of the organ in which the cells are located, not on nuclear structure. The cells of the hematolymphoid system are an exception to this generalization. Hematolymphoid cells with mutually exclusive functions have cytological features that are sufficiently distinctive that they can be readily subclassified. For example, the segmented nuclei of neutrophils and eosinophils (supplemented with the chromatic features of cytoplasmic granules) distinguish these cells from other hematolymphoid cells. The chromatin pattern of lymphocytes is sufficiently characteristic that these cells can be identified in thyroid cytology smears and distinguished from the admixed thyroid follicular cells. Macrophages/histiocytes have distinctive nuclear shapes often likened to a footprint in the sand and a vesicular pattern of chromatin that permits their identification in a complex cellular environment such as the lamina propria of bowel. Plasma cells have a blocked chromatin pattern, consisting of uniformly distributed heterochromatin and colloquially termed "spokes in wheel" pattern. And, megakaryocytes have large multilobated nuclei.

PATHOLOGICAL CELLS WITH DISTINCTIVE NUCLEAR STRUCTURES

There are a relatively limited number of cytologically distinctive changes in nuclear structure of human somatic cells that correlate with cell function and/or disease (Table I). For example, the even spacing of heterochromatin within nuclei of plasma cells is recapitulated in the malignant cells of multiple myeloma. Nuclear grooves, which represent prominent narrow in-foldings of the nuclear membrane, characterize the cells of several otherwise unrelated neoplasms—papillary carcinoma of the thyroid and sex-stromal cell tumors. Clearing of the nuclei, producing a histological appearance that has been nicknamed "Orphan Annie nuclei", characterizes papillary carcinoma of the thyroid. And, the nuclei of proliferating cells and of cells that have been "injured", that is, epithelial cells that are repopulating an injured surface, or cells reacting to "injury", that is, the myofibroblasts associated with healing an ulcer, are characterized by enlarged, round, nucleoli, and an open pattern of chromatin.

The nuclear features that are most profound in terms of relevance to both underlying biological events and to human disease are the changes in cells and nuclei that occur in cancer. The field of diagnostic cytopathology is founded on the premise that the cytologic changes observed in cancer cells are sufficiently distinctive to permit identification of cancer cells as malignant when viewed in isolation from their tissue context. A specific diagnosis of cancer can be made, in the appropriate clinical context, based upon changes seen in as few as a couple of cells. The classical light microscopic cytomorphologic features that lead to a diagnosis of malignancy are predominantly nuclear changes. These features include increased nuclear size (nucleomegaly), particularly an increase in nuclear size out of proportion to the volume of cytoplasm (increased nuclear to cytoplasmic ratio), nuclear membrane alterations including in-foldings/grooves, angulation, and thickening, variability of nuclear size (anisonucleosis), enlargement of nucleoli (nucleolomegaly, macronucleoli), and alterations of the chromatin pattern including dispersal of chromatin, hyperchromasia, and coarsening of chromatin (Fig. 1). These features are recapitulated at the transmission electron microscopic level (Fig. 2).

However, the criteria for diagnosing carcinoma are more nuanced than we have generalized above, varying with both the organ and the

TABLE I. Distinctive Nuclear Shapes of Specific Cell Types

Cell type	Nuclear features
Most carcinoma cells	Enlargement, irregularity and complexity of the nuclear membrane, an irregular pattern of clumping of course heterochromatin and enlarged, pleomorphic nucleoli
Neuroendocrine cells (normal and neoplastic)	Fine clumping of heterochromatin ("salt and pepper" pattern)
Prostate adenocarcinoma	Large, round nuclei with open heterochromatin and prominent nucleoli
Prostate adenocarcinoma (androgen-deprived)	Small, dense nuclei with inconspicuous nucleoli
Plasma cells (benign and malignant)	Regular pattern of chromatin clumping (spoked wheel pattern)
Sex cord-stromal cells	Nuclear grooves
Papillary thyroid carcinoma cells	Nuclear grooves, pseudoinclusions ("Orphan Annie eyes")
Reactive stromal cells	Enlarged nucleoli and a fine chromatin pattern
Neutrophils	Poly-segmented nuclei

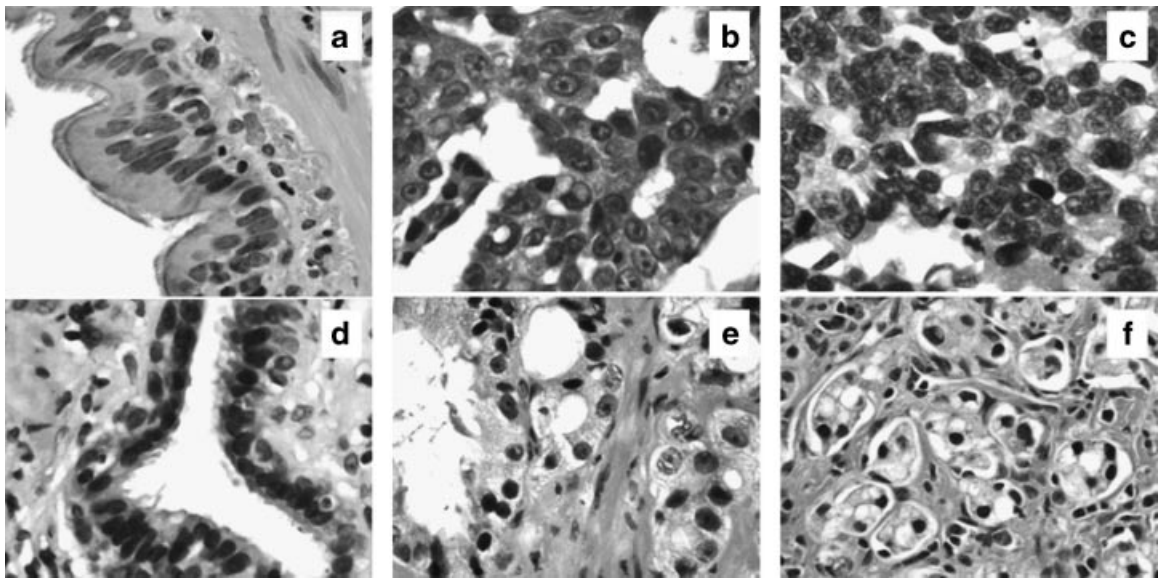

Fig. 1. Lung: (**a**) normal respiratory epithelium, (**b**) non-small cell carcinoma, and (**c**) and small cell carcinoma. Note the prominent nucleoli and coarsely clumped, irregularly distributed chromatin of non-small cell carcinoma (b). In contrast, the chromatin of small cell carcinoma is diffusely distributed and finely clumped (c). Prostate: (**d**) normal epithelium, (**e**) prostate adenocarcinoma, and (**f**) androgen-deprived prostate adenocarcinoma. Note the large, round nuclei with open chromatin and prominent nucleoli of prostate adenocarcinoma that has not been treated with androgen deprivation (e). In contrast, androgen-deprived prostate carcinoma is characterized by small nuclei with dense chromatin and indistinct nucleoli (f).

clinical setting. For example, the two major subtypes of lung carcinoma are non-small cell carcinoma and small cell undifferentiated (or, poorly differentiated neuroendocrine) carcinoma. Distinguishing these two subtypes is critical due to the markedly more rapid rate of progression of small cell carcinoma and the different chemotherapy regimens that are used in treating metastases of these two cancer subtypes. Nuclear chromatin provides the basis for making the diagnosis. Descriptively, the chromatin of small cell carcinoma is diffuse and finely clumped (colloquially termed "salt and pepper" pattern). In contrast, the chromatin of non-small cell carcinoma is coarsely clumped and irregularly distributed (Fig. 1). Carcinoma of the prostate has a different nuclear morphology than that of lung carcinoma. The nuclei are

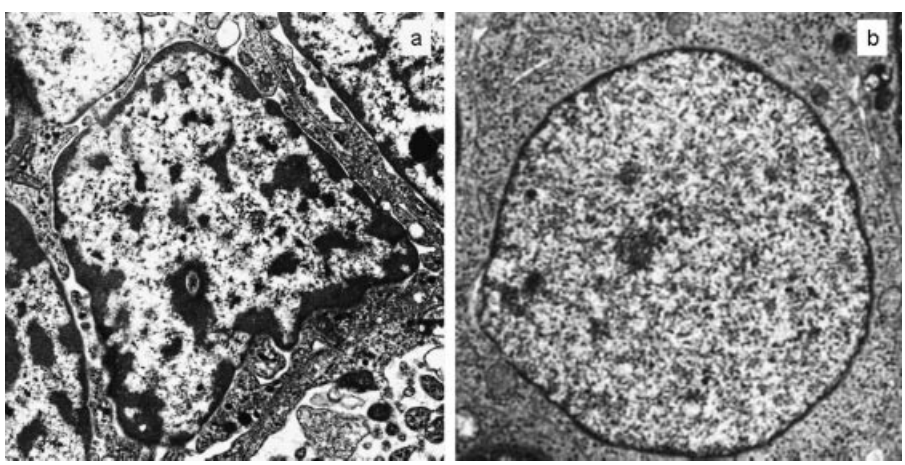

Fig. 2. Transmission electron micrographs of the nuclei of (**a**) a large cell (non-small cell) carcinoma and (**b**) a small cell (neuroendocrine) carcinoma. Note prominent, irregularly distributed heterochromatin in the large cell carcinoma, contrasting with the diffusely distributed, small aggregates of heterochromatin in the small cell carcinoma.

enlarged and round with a clear nuclear background and large, round nucleoli (Fig. 1). This structure differs from that of androgen-deprived (or, hormonally suppressed) prostate carcinoma, which is characterized by "shrunken", small nuclei with dense chromatin and indistinct nucleoli (Fig. 1).

Although the diagnostic cytopathologist provides a diagnosis based on the microscopic appearance of the cells, the structural elements of the cells can also be measured using the tools of morphometry and image analysis. A reflection of how accurately perturbations in the measurable image parameters of diseased cells can provide values that enable one to accurately categorize cells is reflected in the fact that hands-off image analysis systems are now used in many cytopathology labs to classify Pap smears of cervical epithelial cells.

MORPHOMETRY

The microscopically visible components of nuclear structure can be identified and measured using morphometric and image analysis techniques. These techniques fall into three categories—morphometric, densitometric (or, for emitted light, light intensity), and texture. Morphometry characterizes the size and shape of organelles and structures, such as nuclei, nucleoli, nuclear membranes, and chromatin granules. Densitometry (or, light intensity) characterizes the optical density (or light intensity) of structures such as nuclei and nucleoli, or labels of structures, that is, immunoperoxidase and immunofluorescent stains. For example, feulgen binds to DNA; the optical density of feulgen-stained cells directly correlates with the relative amount of nuclear DNA (subject to the effect of chromatin packing or density). Immunoperoxidase stains localize markers, as either optically dense peroxidase substrates or as fluorescent light emitting compounds, to specific molecules. Dyes that bind in a stoichiometric manner to chemical moieties "measure" the amount of those chemical moieties. Although use of feulgen to measure DNA is the best example of this application, immunoperoxidase stains have been widely used to "measure" amount of antigen. The latter approach is of questionable accuracy [True, 1988].

These imaging-based methods characterize the size, shape, and distribution and variance in the parameters of nuclear structures that can be identified, typically at the light microscopic level. The organelles that are thus evaluated include the nucleus, nuclear membrane, nucleolus, and heterochromatin. Combinations of parameters can be used to evaluate the "texture" of internal structures and, hence, measure aspects of the organization of the DNA and DNA-associated proteins in chromatin. Texture reflects the distribution of objects, that is, the distribution of gray levels within a hematoxylin-stained nucleus reflects the distribution of optically dense heterochromatin within the nucleus.

MORPHOMETRIC ANALYSIS OF CANCER NUCLEI

According to Doudkine, and exemplified by Baak [1991] for a wide range of cancers, texture features are useful for characterizing cancer nuclei and for distinguishing cancer from normal cells of the organ in which the cancer originated. Features descriptive of nuclear structure can be categorized as follows: descriptive statistics that characterize chromatin distribution; texture features; the extreme values of range of parameter values; Markovian features; run length values; and fractal texture features [Doudkine et al., 1995]. As will be discussed below (in "Molecular Basis of Nuclear Changes") the pattern of chromatin in nuclei reflects changes in distribution, translocation, and activation of genes or sets of genes. Many tumors are characterized by variably extensive changes in the genome. Presumably these changes underlie, at least in part, the dramatic changes in cancer nuclei that are viewed by the cytopathologist.

EXAMPLES OF DISTINCTIVE CANCER NUCLEI

Morphometric features provide a basis for subcategorizing lung carcinomas. Chromatin texture distinguishes small cell versus non-small cell carcinoma of the lung [Thunnissen et al., 1992]. Although greater than 100 morphometric parameters can be used to uniquely characterize the nuclear structure of cancer cells, the two subtypes of lung carcinoma can be distinguished using only four parameters, which describe the compactness and distribution of chromatin—mean harmony, standard deviation of gray level, standard deviation of run length, and standard deviation of run percent [Schmid et al., 2006].

Additional examples of how chromatin texture can be used to distinguish histopathologic subtypes of tumor include urothelial carcinomas of the urinary tract of different grade [van Velthoven et al., 1995], follicular epithelial neoplasms of the thyroid [Liautaud-Roger et al., 1992], glandular neoplasms of the colon [Mulder et al., 1992] and breast carcinomas of different grade [Poulin et al., 2003]. The morphometric parameters that distinguish these tumor types differ by organ. Thyroid follicular adenomas differ from follicular carcinomas with respect to the skewness of optical density histograms of stained nuclei and the standard deviation of the optical density measurements. A single chromosomal translocation is associated with these changes [Liautaud-Roger et al., 1992]. Increases in nuclear area and in the nuclear shape factor, which measures out-of-roundness of nuclei, distinguish colonic adenoma from adenocarcinoma of the colon [Mulder et al., 1992]. The morphometric parameters that correlate with and contribute to the basis for grading breast carcinoma are nuclear size and shape and chromatin distribution [Poulin et al., 2003].

SUBVISUAL CHANGES IN DISEASE-ASSOCIATED CELLS

The ability to precisely measure objects using morphometric and densitometric techniques raises the prospect of detecting features that are too subtle for the human eye to detect. This is true for a number of organs. There is long-standing evidence of changes in the histology of light microscopically "normal" cells from an organ that harbors a cancer. Although the changes are too subtle to consistently discern microscopically, they are sufficiently constant that they can be measured morphometrically. For example, light microscopically normal squamous epithelial cells from the cervix of patients with cervical intraepithelial neoplasia (CIN) exhibit changes in nuclear DNA texture that overlap with the features of the CIN cells but that are undetectable by the human eye [Bibbo et al., 1981]. In the prostate, nuclear features distinguish histologically normal luminal epithelial cells from prostates of patients with an adenocarcinoma elsewhere in that prostate from the corresponding cells in prostates presumed not to have a cancer from which tissue was removed to treat benign prostatic hyperplasia. A discriminator consisting of three nuclear texture features that separate benign from cancer cases was derived from a training set of cases, and then applied to an independent set of test cases [Mairinger et al., 1999].

The same conceptual approach was used to evaluate cervical epithelial cells for infection by human papilloma virus (HPV). Changes in the organization of chromatin—the chromatin texture—correlated with HPV infection and raised the prospect of using nuclear shape and texture parameters to identify HPV-infected squamous cells [Guillaud et al., 2005].

Since anatomic pathologists base their diagnoses on two-dimensional structures, one would predict that features of cells analyzed in three dimensions would provide a more accurate classification than is obtained from 2D images. Using confocal microscopy Huisman et al. [2005] found a set of nuclear texture features that discriminated between benign and malignant prostate epithelial cell nuclei. Contrary to the anticipation that 3D information would more accurately distinguish these nuclei than 2D-based parameters, classification success was only modest. Only 76% of benign nuclei and 92% of cancer nuclei were correctly classified [Huisman et al., 2007].

This relatively poor classification rate raises the question of whether the human eye is the appropriate "gold standard". In other words, are the 24% of benign cells that Huisman "misclassified" as malignant truly benign; or might they be a potentially metastatic subpopulation of cells that only appear histologically "benign"? Conversely, are the 8% of malignant nuclei that were "misclassified" as benign truly benign, or might they be a better differentiated, less potentially invasive and metastatic subpopulation of cells? This and other studies raise additional questions. Is there a set of molecular markers that more precisely categorizes cells and predicts clinical outcome with higher precision than either cytopathological or morphometric markers? Can structural abnormalities of nuclei provide a basis for selecting cells for discovering these markers? A test comparing the specificity of morphometry and vision-based classification has not been done. The fact that subvisual changes in cells can be detected in cells that appear "normal" to the human eye argues that machine vision may be a more precise classifier.

Although subvisual abnormalities are of potential clinical value, they have neither been employed for clinical decision making nor have they been used to provide a basis for understanding molecular mechanisms. Several explanations come to mind. One is that morphometry is such a time-consuming method subject to multiple sources of variance that little confidence or attention has been placed on it. A second possible explanation lies in the fact that the three communities involved with the analysis of the structures of single cells—diagnostic cytopathologists, morphometrists, and molecular biologists—do not routinely communicate with one another. Consequently the potentials and limitations of respective fields of knowledge are little known and are infrequently shared.

PROGNOSTIC FEATURES

There is also evidence that nuclear structure of different cancers provides prognostic information. Coarse nuclear texture of adenocarcinomas of the colon, which is inversely correlated with fractional allelic loss, predicts cancer recurrence [Mulder et al., 1992]. The out-of-roundness of prostate carcinoma nuclei correlates with progression, providing, in one study, better correlation with clinical outcome than tumor grade [Epstein et al., 1984]. Nucleoli in higher-grade prostate carcinomas tend to be more peripheral than nucleoli of lower grade tumors [Helpap, 1988]. Variance, or heterogeneity, of expression of immunoreactive androgen receptor by prostate carcinomas inversely correlates with response to androgen deprivation therapy and time to disease progression [Sadi and Barrack, 1993]. Tumors that respond to hormonal therapy have a more uniform distribution of androgen receptor immunoreactivity. As a final example, nuclear shape, which is one of the components of breast cancer grade, predicts breast cancer progression [Poulin et al., 2003].

SOURCES OF ERROR IN MORPHOMETRY

Morphometry is subject to multiple different sources of error [True, 1996]. Some examples are:

- Tissues shrink during fixation in formalin solutions. Shrinkage can disproportionately change the volume fractions of tissue components. And, the degree of shrinkage varies with the type of tissue [Collan et al., 1987b].
- Scale of analysis can influence the absolute values of linear measurements ("coast of England" effect). For example, complexity of surfaces results in increased lengths of these surfaces at progressively higher magnifications [Mandelbrot, 1975].
- Sections of optically dense structures that are of significant size can lead to an overestimate of the volume fraction of these structures (Holmes effect). If the section is of greater than infinitesimal thinness compared with the optically dense object, the sampling will not represent the size of the object, but will be biased toward a greater than representative size [Collan et al., 1987a].
- Recognition of these, and other, sources of error can be dealt with, in part, by attention to sampling and, sometimes, by increasing sample size.

SAMPLING

What may be the largest and often overlooked source of error is the sampling strategy. Examples of how distribution of events can lead to sampling errors follow:

- The cells at the periphery of many carcinomas are in the proliferative phases of the cell cycle [Shoji et al., 1999]. The nuclei of proliferating cells enlarge as DNA is duplicated.
- Nuclei from areas of extraprostatic invasion by prostate carcinoma are less round than those from the corresponding intraprostatic portion of the tumor. Cells sampled from the periphery of organ-confined tumors have a greater nuclear roundness factor than did those sampled from the center or randomly throughout the tumor [Mohler et al., 1994a].
- Location in a specimen and the type of specimen has an apparent effect on nuclear size. For example, prostate cancer nuclei in biopsies are both smaller and have a more abnormal shape than do cells in the corresponding prostatectomy specimens [Mohler et al., 1994b].

- The selection of the field of view to analyze is subject to observer bias, as is the choice of a threshold that distinguishes objects of interest form other objects and from "background."

Relevant to sampling is the fact that most measurements are based on two-dimensional images of three-dimensional structures. Few 3D analyses have been done. Furthermore, not only are living cells best viewed in three dimensions, but there is a significant temporal domain, often not considered, to any analysis of nuclear structure. Some nucleus-based molecular events occur within minutes, such as the binding and release of components of transcription complexes to DNA and cell replication events [Becker et al., 2002; Phair et al., 2004]. Analysis of nuclei at one time point fails to catch such phenomena. Only if we analyze large numbers of cells, where the number of cells is sufficient to represent temporally rare events, can we hope to identify nuclear events of such a transient nature. Even normal cells in different physiologic states, such as the stage of the cell cycle, differ in measurable nuclear features. For example, nuclei with 4N chromosomal content, which are on the verge of dividing, have larger nuclei and more DNA and chromatin than do cells at earlier stages in the cell cycle.

EXCEPTIONS TO GENERALIZATIONS REGARDING THE NUCLEAR STRUCTURE OF CANCERS

Although, as a generalization, most cancer nuclei have a predictable microscopic phenotype—nucleomegaly, pleomorphism, poikilonucleosis, nucleolomegaly, coarseness, and clumping of chromatin—some cancer cells have very benign appearing nuclei. This is true not only of carcinomas that have received systemic therapy, that is, androgen deprivation therapy of prostate carcinoma, but also of histologic variants of common carcinomas. The nuclei of lobular carcinoma of the breast and the transition zone variant of prostate adenocarcinoma are small and round with inconspicuous nucleoli and uniformly dense heterochromatin. These cancers are diagnosed based on abnormal architecture and clinical context rather than their nuclear structure. Conversely, some benign processes have nuclear features characteristic of many cancers—large nucleoli, clumped chromatin—reactive fibroblastic processes, which may form masses (termed "pseudotumors") and injury, for example, radiation injury to the prostate luminal epithelial cells.

BIOCHEMISTRY OF NUCLEAR STRUCTURE

Normal and neoplastic nuclei have been characterized at an ultrastructural level using methods that extract DNA, RNA, and soluble, low molecular weight proteins, leaving insoluble material that appears to form a nuclear skeleton that presumably has a structural and, perhaps, functional role. Termed the nuclear matrix [Penman, 1995], these insoluble proteins have been extracted from carcinomas of different origin. Proteins differentially expressed by normal and malignant tissues of specific organs have been used to generate antibodies, with the expectation that these antibodies can be used to screen patients for recurrent cancer. For example, urine samples from patients with a history of urothelial carcinoma have been screened for matrix-associated protein NMP-22 [Getzenberg et al., 1996]. Identifying the molecular components that distinguish the nuclear matrix of cancer cells from normal cells provides the basis for better understanding the molecular pathways of cancer [Nickerson, 1998].

However, the existence of a nuclear matrix has been questioned [Pederson, 2000]. Arguments against the existence of a matrix include the absence of an identifiable filamentous network throughout nuclei and the structure of nuclear interchromatin spaces, which, being sinusoidal and localized, are thought not to provide a space for trans-nuclear filaments. Hancock [2004] argues that the concentrations of macromolecules are sufficient to create a highly organized, complex structure that suggests, in its high degree of order, an underlying nuclear matrix, despite there not necessarily being one. Furthermore, the proposal that there is an extensive nuclear matrix would need to be reconciled with evidence that the nucleus consists of compartments of specific chromosomes and associated proteins. As a reconciliation, Martelli et al. [2002] have proposed that there are compartment-specific matrices. A few specific nuclear molecular elements have been identified. These include non-myogenic nuclear actin [Pederson and Aebi, 2005], NuMA, nuclear lamins, and nuclear pore associated proteins Nup 153 and Tpr. However, the roles of

these proteins are unknown [Pederson, 2000; Grummt, 2006].

NUCLEAR COMPARTMENTS

Correlation of light and ultrastructural transmission microscopy with tissue-localization techniques of in-situ hybridization and immunohistochemistry has demonstrated compartments within nuclei. These compartments contain distinct sets of chromosomes and associated proteins. Although there is a large and growing literature regarding the elements of nuclear compartments [Cremer et al., 2004; Kosak and Groudine, 2004; Meaburn et al., 2007; Misteli, 2007], a brief listing of non-chromosome elements follows (see also Table II):

- Nucleoli are composed of ribosomal RNA and provide a site for assembly of rRNA subunits. A recent observation is that nucleoli appear to move within the nuclear space [Fung and De Boni, 1988].
- Heterochromatin is condensed chromatin material that appears to lack significant transcription activity and is gene-poor. Heterochromatin is electron dense at the ultrastructural level and is densely hematoxyphilic at the light microscopic level.
- Perichromatin domains, which are adjacent to heterochromatin, are sites of transcription; the earliest assembled portions of transcripts appear as perichromatin fibers. Also within perichromatin domains are transcription factors and RNA processing molecules.
- An interchromatin compartment lies between heterochromatin. This contains "open", or euchromatin domains, which are gene-rich. However, the genes in this compartment are not necessarily actively transcribed [Gilbert et al., 2004].
- The Cajal (coiled) body is a domain where RNA processing factors accumulate [Frey and Matera, 2001].
- Speckles, or interchromatin granules, are electron dense structures that appear to be the site for storage of transcription factors and assembly of splicing complexes [Lamond and Spector, 2003; Misteli, 2007].
- Promyelocytic leukemia bodies (PML bodies) are small, nuclear matrix-associated structures. Proteins in PML's appear to have a role in activating promoters [Boisvert et al., 2001; Rivera et al., 2003; Hancock, 2004].
- Insulators are hypothesized structures that bound compartments [Capelson and Corces, 2004].

Much recent work characterizes in successively greater detail functions of these structures and compartments. For example, to allow RNA polymerase to transcribe genes chromatin is remodeled into euchromatin (transcription-susceptible) and heterochromatin (transcription-incompatible) structures [Percipalle and Farrants, 2006]. The role of the putative nuclear matrix in the functionality of nuclear elements is uncertain.

MOLECULAR BASIS OF NUCLEAR CHANGES

Some of the molecular elements of specific nuclear shapes have been characterized in part. For example, the distinctive multilobed shape of neutrophils is associated with reduced lamin content, which appears to increase the flexibility of the nuclear envelope and facilitate connections to the heterochromatin [Olins and Olins, 2004]. Specific chromosome domains appear to localize to different lobes of neutrophils nuclei [Bartova et al., 2001].

TABLE II. Elements of Nuclear Structure

Non-chromosome structure	Comment
Nucleoli	Assembly of RNA
Heterochromatin	Gene-poor
Perichromatin domain	Transcription site
Interchromatin compartment	Gene-rich
Cajal body	Site of RNA processing factors
Speckles	Storage of transcription factors and assembly of splicing complexes
PML bodies	Promoter activation
Insulators	Structures that bound nuclear compartments

Many molecular events have been localized to nuclear regions at a high level of spatial resolution. For example, androgen receptor location within the nucleus depends on agonist-induced activation [Tomura et al., 2001]. X chromosome-linked genes are organized in the nuclear space in a manner so that they are carefully positioned with respect to activation status [Clemson et al., 2006]. And, the position of BRCA1 in the nucleus appears to have a functional role in regulation of XIST [Pageau et al., 2007].

A CHALLENGE

The identification of molecules that are specific to nuclear elements provides the basis for understanding their role in the structure of cancer cell nuclei. Despite increasing detail with respect to precise localization and characterization of molecular elements of nuclear function, our knowledge of the molecular architecture of cancer nuclei is rudimentary at best. Consequently, we can pose some basic questions that might direct future research. Is the chromatin of cancer cell nuclei so scrambled that the pleomorphic cells viewed by the cytopathologist are an epiphenomenon? In support of this possibility is the observation that carcinomas have very complex karyotypes, of which very few are clonal. Are those cells that are so microscopically distinctive and that provide the cytologic basis for a cancer diagnosis not the potentially proliferative-metastatic cells but, rather, end-stage cells lacking malignant functionality? And, conversely, are the malignant cells cytologically indistinct? Alternatively, is transcription of genes in cancer cells so altered in spatial distribution that the consequent changes can be readily recognized by light microscopy? Does spatial derangement promote additional genetic/transcriptional abnormalities? Are spatial derangements additional "hits" in the progression of the neoplastic state? Can the nuclear matrix be targeted pharmaceutically in such a way to normalize cellular function and/or proliferation of neoplastic cells? May these changes be observed in the cytomorphologic phenotype of treated cells?

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