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Nuclear Microenvironments in Cancer Series

PROSPECTS

Nuclear Microenvironments in Cancer Diagnosis and Treatment

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Abstract The nuclear architecture plays an important role in the temporal and spatial control of complex functional processes within the nucleus. Alterations in nuclear structures are characteristic of cancer cells and the mechanisms underlying these perturbations may directly contribute to tumor development and progression. In this review, we will highlight aspects of the nuclear microenvironment that are perturbed during tumorigenesis and discuss how a greater understanding of the role of nuclear structure in the control of gene expression can provide new options for cancer diagnosis and treatment. J. Cell. Biochem. 104: 1953–1963, 2008. © 2007 Wiley-Liss, Inc.

Key words: nuclear architecture; cancer; gene expression

The nuclear microenvironment plays an important role in organizing many components of the gene regulatory machinery. The intact architecture of the interphase nucleus is essential for the coordinated temporal and spatial regulation of complex biological processes from DNA synthesis to transcription and protein expression. In human cancers, we can see how even small changes in the organization of the nuclear microenvironment can lead to significant perturbations in the regulation of gene expression resulting in aberrant cell growth and differentiation. Treatment of tumors with chemotherapeutic drugs often restores the normal nuclear structure and function [Zink et al., 2004b]. New technologies for visualizing the molecular components of the nucleus in intact cells have provided much insight into the spatial and temporal organization of gene expression and have led to a greater understanding of both normal cellular physiology and how derangements in nuclear architecture result in various pathological states.

Perhaps one of most common examples of the use of nuclear structural features to aid in the diagnosis of cancer is the papanicolaou test in which a special stain applied to epithelial cells from the cervix is used to detect cervical adenomas and their precursors. As the grade of the lesion increases, there is a change in the sizes and shapes of nuclear and cytoplasmic structures and an increase in the nucleus to cytoplasm ratio reflecting a loss of differentiation [DeMay, 1996].

Cancer cells contain many structural abnormalities in their nuclei and these alterations are thought to play an important role in cancer development and progression. In this review, we will focus on the components of the nucleus that have a regulatory role known to be altered in human cancers (Fig. 1): (1) nucleoli, (2) promyelocytic leukemia (PML) bodies, (3) chromosomes, and (4) sites of transcription. We will begin with a discussion of the structure and function of each of these components with an emphasis on how they may be altered in

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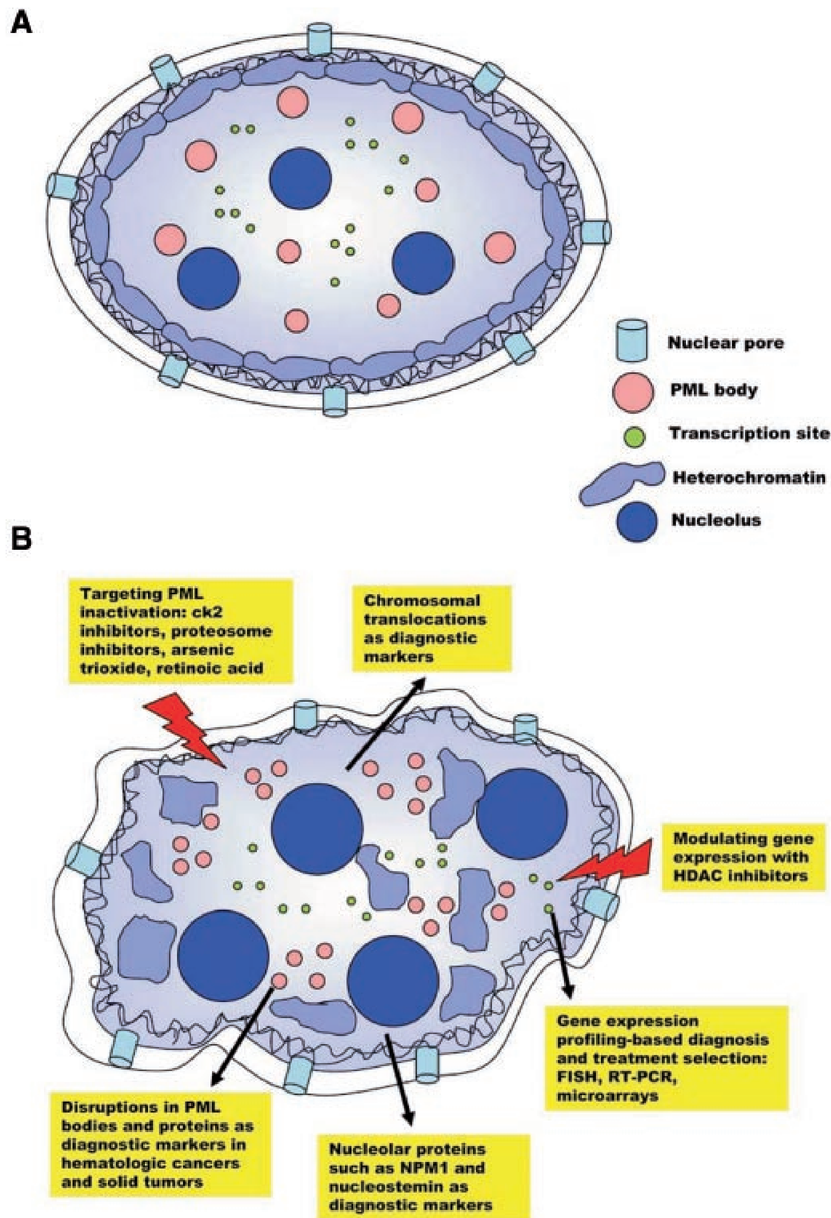

Fig. 1. Nuclear architecture and organization of functional components in normal and cancer cells. **A**: Nucleus of a normal cell. The double-membrane nuclear envelope contains pores and is connected on its inner surface to the nuclear lamina meshwork. Bound to the nuclear lamina is chromatin, with areas of heterochromatin located along the nuclear periphery and euchromatin located in the nuclear interior. Other structures present within the nucleus include PML bodies, nucleoli, and sites of transcription. Only those nuclear components discussed in this review are shown in the figure. **B**: Nucleus of a cancer cell. Some structural alterations observed in cancer cells include heterochromatin aggregates, enlarged nucleoli, and disrupted PML bodies. This figure emphasizes specific nuclear components of interest in the diagnosis and treatment of cancer, as discussed in this review. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

cancer cells and then analyze how recent studies have revealed mechanisms linking nuclear structure and gene expression and may provide novel approaches for more accurate cancer diagnosis and more targeted therapies.

THE NUCLEOLAR COMPARTMENT

The nucleolus is the nuclear structure where ribosomal RNA transcription and ribosome assembly take place. One of the earliest observations in cancer cells was the alteration in

numbers and sizes of nucleoli. In addition to its function in ribosome biosynthesis, the nucleolus may also function as a stress sensor for the cell, playing a role in the stabilization of specific proteins and thereby modulating the molecular pathways in which these proteins are involved. Many nucleolar proteins are mutated, overexpressed, rearranged, or deleted in human cancers, emphasizing the important role the nucleolus plays in tumorigenesis. For example, in response to cellular stress, such as UV irradiation, nucleotide depletion, heat shock, or hypoxia, the nucleolar tumor suppressor protein ARF is upregulated causing nuclear retention of the oncoprotein Mdm2 which is a negative regulator of p53 [Weber et al., 1999; Rubbi and Milner, 2003]. This results in cell cycle arrest and stabilization of p53.

Mutations of the nucleolar protein nucleophosmin (NPM1) are characteristic of many acute myeloid leukemias [Falini et al., 2005] and lead to delocalization and destabilization of ARF. Expression of a nucleophosmin mutant which localizes mainly to the cytoplasm reduces the ability of ARF to initiate a p53 response and to induce cell cycle arrest [Colombo et al., 2006]. By targeting the mutant nucleophosmin to its correct location in the nucleolus, it may be possible to induce p53-mediated apoptosis in leukemic cells. Inactivation of NPM1 was found to cause genomic instability and NPM1 heterozygous mice which also contained mutations in c-myc had earlier onset of B-cell lymphomas when compared to mice with normal NPM1 [Grisendi et al., 2005]. This indicates that NPM1 mutations may cooperate with mutations in other oncogenes to accelerate tumorigenesis and identifies NPM1 as a possible therapeutic target in cancers in which it is mutated. Further, since NPM1 is overexpressed in many cancers including gastric, colon, ovarian, and prostate carcinomas, it may be useful as a marker for the presence of tumor cells in these tissues [Grisendi et al., 2006]. As a diagnostic marker, NPM1 would be important in distinguishing subtypes of AML with distinct molecular, pathological and clinical features [Falini et al., 2007] and would also be a good target for the development of new chemotherapy drugs, useful in the treatment of leukemias. Changes in the expression level of NPM1 may also be correlated with tumor progression and thus, it may be a useful marker in monitoring response to chemotherapeutic treatment or disease recurrence after therapy. Furthermore, since mutant NPM1 mislocalizes to the cytoplasm and the regression of tumors may correlate with movement of NPM1 back to its correct compartment in the nucleolus, the response of cancers to treatments targeting NPM1 could be monitored by immunofluorescence in tissue biopsies.

Another p53 binding protein, nucleostemin is also localized to the nucleolus but only found in stem cells and cancer cells, not in committed and terminally differentiated cells [Tsai and McKay, 2002]. Drugs that promote the nucleolar retention of nucleostemin may be effective in preventing the degradation of p53, leading to increased apoptosis and thus may be used as targeted therapies for cancers that misexpress nucleostemin. These findings suggest that in tumorigenesis, cells may increase expression of nucleolar proteins such as nucleostemin leading to dedifferentiation and increased cell proliferation. Alternatively, perturbation of nucleolar structure during tumor development may interfere with the role of the nucleolus as a stress sensor and lead to an increase in the activity of anti-apoptotic proteins.

The nucleolus downregulates ribosomal RNA synthesis through the transcription factor TIF-IA, which modulates the activity of RNA Pol I [Schnapp et al., 1990]. Cellular stress causes the phosphorylation and inactivation of TIF-IA by c-Jun N-terminal kinase 2 (JNK2) leading to impaired transcription complex formation and a decrease in rRNA synthesis [Mayer et al., 2005]. In mouse embryonic fibroblasts, Cre-mediated inactivation of TIF-IA caused nucleolar disruption, cell cycle arrest, and upregulation of p53 and apoptosis [Yuan et al., 2005]. A further role for the nucleolus in the regulation of cell growth and apoptosis was shown by another study in which inhibition of the degradation of the c-myc oncoprotein resulted in c-myc accumulation in nucleoli, binding of c-myc to rDNA and stimulation of transcription by RNA Pol I [Arabi et al., 2005]. Together, these studies indicate that the nucleolus is a dynamic compartment with a key role in the cellular response to stress.

The alteration of nucleolar structure observed in cancer cells, thus, has important consequences for its role as a regulator of p53 activity. In cancer, impairment of normal nucleolar function may thus lead to decreased nucleolar retention of Mdm2 and increased degradation of p53 in the nucleoplasm resulting

in decreased apoptosis. On the other hand, mutations in oncogenic proteins such as c-myc may interfere with normal nucleolar function and lead to increased cell proliferation. The observation of larger and more prominent nucleoli in tumor cells may thus reflect not only increased ribosomal RNA synthesis but also changes in expression of nucleolar proteins involved in the control of differentiation and apoptosis.

PML BODIES

PML bodies are nuclear structures that contain the tumor suppressor PML and many other proteins including the tumor suppressors RB and p53 [Fogal et al., 2000; Guo et al., 2000; Zhong et al., 2000; Bernardi and Pandolfi, 2003]. PML protein has been linked to various biological processes including growth arrest, senescence, and apoptosis and is also thought to be involved in transcription, DNA repair and proteolysis [Lallemand-Breitenbach and de The, 2006]. In response to DNA damage, PML was shown to enhance p53 stability by sequestering the p53 ubiquitin ligase Mdm2 in the nucleolus, preventing p53 degradation and thus increasing its activity [Bernardi et al., 2004]. This study showed that in PML-/- cells sequestration of Mdm2 in the nucleolus and stabilization of p53 was impaired resulting in decreased apoptosis. Furthermore, studies in PML knockout mice have shown that PML is essential for multiple apoptotic pathways [Wang et al., 1998]. In tumors with p53 mutations, there may be increased probability of PML loss since PML was shown to be transcriptionally controlled by p53 [de Stanchina et al., 2004].

In acute PML, translocation between the PML gene on chromosome 15 and the retinoic acid receptor α gene (RARα) on chromosome 17 results in a PML–RARα fusion protein [Borrow et al., 1990; de The et al., 1990] that causes normal PML protein to become mislocalized and disrupts normal PML bodies [Melnick and Licht, 1999]. This results in de-differentiation and proliferation of the leukemic cells. The observation that PML bodies are disorganized in acute PML cells provided early evidence that the alteration of nuclear structure may play a role in human cancer. Treatment of acute PML with all-trans retinoic acid [Huang et al., 1988] or arsenic trioxide [Zhu et al., 1997] restores normal PML body structure and function through a mechanism involving the degradation of the PML–RARα fusion protein [Zhu et al., 1999, 2001].

PML may also have a role in the development of other cancers, since it is aberrantly expressed in various solid tumors. For example, in tumors of the colon, prostate, lung, breast, and central nervous system, PML expression was found to be reduced or absent [Gambacorta et al., 1996; Zhang et al., 2000; Gurrieri et al., 2004]. It is possible that a decrease or loss of PML protein in such tumors may promote tumor formation and recent studies may shed new light on the functional consequence of PML loss of human tumors. In the PTEN-/- mouse model in colon cancer, loss of PML was correlated with the accelerated growth of polyps and tumors and PML deficiency alone was shown to increase tumorigenesis in the prostate by specifically recruiting nuclear phosphorylated Akt into PML bodies [Trotman et al., 2006]. This indicates that PML loss has an important role in the network of nuclear tumor suppressors that oppose the oncogenic kinase Akt. The role of PML in accelerating tumor development and progression after PTEN loss is especially relevant in cancers of the prostate and colon, which often have mutations in the PTEN tumor suppressor pathway. Together with mutations in critical tumor suppressors, decreased expression or loss of PML in tumors may therefore lead to a more aggressive tumor phenotype. The observation that PML bodies contain proteins involved in many cellular processes such as growth arrest, apoptosis, and tumor suppression indicates that proper compartmentalization of certain nuclear proteins within PML bodies plays an important role in preventing tumorigenesis and links changes in the structural organization of nuclear compartments with functional changes in nuclear proteins.

Inactivation of PML has recently been shown to lead to increased tumorigenesis in a Ras-induced transgenic mouse model of lung cancer [Scaglioni et al., 2006]. In this study, it was shown that PML protein levels are regulated by its phosphorylation by casein kinase 2 (ck2), a kinase often activated in human cancers, and subsequent proteasome degradation. Further, PML and ck2 levels were found to be inversely correlated in human tumor cell lines. Pharmacologic inhibition of ck2 restored PML function [Scaglioni et al., 2006]. Thus, ck2 inhibitors and proteasome inhibitors may be effective in

treating cancers with perturbations in PML expression.

Together the findings that PML has a direct role in stabilizing apoptotic pathways and that it is mislocalized or reduced in expression in multiple cancers directly link PML with a role in tumorigenesis. The above studies suggest that PML must be intact and localized to a specific nuclear compartment in order for its proper function in regulating apoptosis and suppressing tumorigenesis. Restoring normal PML activity through targeted therapy could be effective in treating many other cancers in which decreased expression of PML is observed.

The example of PML directly links perturbations in nuclear architecture with cancer development and progression and provides an opportunity for the molecular diagnosis of cancer using structure-based assays such as fluorescence in situ hybridization (FISH). Screening tumors for disruptions in PML bodies could identify subtypes of tumors that would respond to new targeted therapies and would also be valuable in monitoring patients for response to treatment and for cancer recurrence. Also new chemotherapy drugs could be tested in a high throughput way based on their ability to cause the reformation of normal PML bodies.

THE SPATIAL ORGANIZATION OF CHROMATIN IN AN INTERPHASE NUCLEUS

Heterochromatin aggregates are a typical feature of many tumor cells but changes in the spatial organization of chromatin within the interphase nuclei of tumor cells have not been as readily observed [Dimitrova and Berezney, 2002; Cremer et al., 2003]. However, the spatial positioning of chromosomes in normal cells is known to play an important role in the development of chromosomal translocations characteristic of many tumors.

Visualization of nucleic acids and proteins in situ has revealed that individual interphase chromosomes tend to occupy distinct territories within the nucleus and have an important role in the activation or suppression of gene expression [Cremer et al., 2006]. The regulation of gene expression and nuclear structure is controlled both by the location of a gene within chromatin and by the subnuclear localization of the gene [Kosak and Groudine, 2004]. Actively transcribed genes are known to be associated with decondensed euchromatin and localized near the center of the nucleus while transcriptionally silent genes are localized near the nuclear periphery within more condensed heterochromatin [Lanctot et al., 2007]. For example, the immunoglobulin heavy chain (IgH) gene locus is localized to the nuclear periphery and suppressed in hematopoetic progenitors before it is activated during B-cell development [Kosak et al., 2002]. On the other hand, the β globin gene relocates away from heterchromatin when activated in erythroid cells [Francastel et al., 1999].

It is still unclear whether the positioning of chromosomes is a cause or effect of gene function. It is also not known, how the spatial organization of chromosomes becomes altered in cancer cells or what effect chromosomal aberrations have on the positioning of genes and their activation or suppression. In cancer, the spatial organization of chromatin and associated gene loci may play a key role in the development of tumors associated with chromosomal translocations. During tumor development, oncogenes may be able to direct the positioning of chromosomes and thus cause the transcriptional activation of genes promoting cell growth and proliferation and silencing other genes that promote apoptosis.

In a study comparing the positions of the gene-poor chromosome 18 and gene-rich chromosome 19 in tumor cells, it was observed that the positioning of chromosomes was altered even with no changes in karyotype [Cremer et al., 2003]. However, this study was performed in cultured cell lines and an examination of cells within tumor tissue may give a more accurate picture of the positioning of chromosomes in tumor cell nuclei. It has also been proposed that the positioning of genes within regions of chromatin may be regulated at the level of individual genes [Zink et al., 2004a]. This study examined the nuclear positioning and transcriptional regulation of four adjacent genes on chromosome 7 in human nuclei and found that inactive genes preferentially associated with heterochromatin in the nuclear periphery while active genes relocated to areas of euchromatin in the nuclear interior. Furthermore, they showed that inhibition of transcription did not affect the perinuclear localization of these genes, suggesting that it is the activation of transcription that causes the movement of a gene to the nuclear interior. The finding that

these genes first became active and then moved to the nuclear interior suggests that the organization of the nucleus may be regulated at the level of individual genes. To better address this question, it will be necessary to examine additional cell lines and genes. It may be possible that for some genes, they first become activated and recruit the transcriptional machinery and then move towards the center of the nucleus to continue transcribing, while in other cases, inactive genes move to the nuclear interior where active genes are localized and then become activated.

A recent study of the β globin gene in murine erythroid cells provides support for the first possibility. Expression of the β globin gene was observed to begin at the nuclear periphery and movement of the gene to the nuclear interior was accompanied by an increase in transcription of the β globin gene [Ragoczy et al., 2006]. This suggests that transcriptional activation of a gene locus is necessary before the gene locus can relocate to the nuclear interior and also provides evidence that transcription can occur in the nuclear periphery in mammalian cells.

Changes in the functional organization of chromosomes are indicative of perturbations in the gene regulatory machinery and can be correlated with the onset and progression of human cancer. Chromosomal aberrations are a typical finding in many tumors and the detection of chromosomal translocations is particularly important in the diagnosis and prognosis of hematologic malignancies. Certain leukemias and lymphomas are often characterized by their specific translocation and the frequency with which these translocations occur may be the result of the spatial organization of the chromosomes within the nucleus.

In many B-cell lymphomas, there are characteristic translocations that occur involving the MYC, BCL, and immunoglobulin loci [Bartova et al., 2000; Willis and Dyer, 2000; Harris et al., 2001; Siebert et al., 2001]. An examination of the spatial proximity of these genes using DNA FISH in normal B-cells showed that loci for MYC and the IgH chain, two genes that are frequently disrupted in Burkitt's lymphoma, were more frequently found together than other rare translocation partners; similarly BCL2 and the IgH chain were often found in close proximity [Roix et al., 2003]. Furthermore, an analysis of the radial positioning of these gene pairs showed that loci observed in close spatial proximity are often localized to the center of the nucleus [Roix et al., 2003]. Thus, the spatial organization of chromosomes within the nucleus determines the probability that two chromosomes will join together to form a chimeric chromosome and contribute to the chromosomal rearrangements that often lead to tumorigenesis [Misteli, 2004].

A recent study in interphase mammalian nuclei emphasizes the role of spatial proximity of chromosomes in translocations and transcription-dependent associations. The degree of intermingling between chromosomes pairs was found to be significantly correlated with translocation frequency, providing additional support for the idea that spatial proximity determines translocation frequency [Branco and Pombo, 2006]. Further, it was observed that inhibition of transcription affected the degree of intermingling between pairs, suggesting that transcription plays a role in the spatial positioning of chromosomes [Branco and Pombo, 2006].

Traditionally, cancer has been treated by blocking cell proliferation non-specifically. The development of drugs targeted to specific structural abnormalities in cancer cells has already led to more individualized treatments tailored to the tumor and patient. In the example of chronic myelogenous leukemia (CML), the observation of a specific chromosomal translocation has led to the development of a selective chemotherapeutic agent which targets cells with this particular structural abnormality. The Philadelphia chromosome (Ph), formed by the translocation of chromosomes 9 and 22 is the hallmark of CML and is also common in acute lymphoblastic leukemia (ALL). Translocation of chromosomes 9 and 22 results in a chimeric gene encoding BCR-ABL1 that encodes the BCR-ABL1 fusion protein [Melo et al., 1993; Hochhaus et al., 1996]. This translocation causes constitutive activation of ABL tyrosine kinase activity which is crucial for leukemogenesis in CML and Ph positive ALL [Deininger et al., 2000]. The selective inhibition of the BCR-ABL1 tyrosine kinase activity by Imatinib mesylate is one of the most effective therapies for CML [Goldman and Melo, 2003]. As a drug which selectively blocks proliferation and induces apoptosis of tumor cells [Druker et al., 1996; Deininger et al., 1997; le Coutre et al., 1999], Imatinib is an example of a targeted chemotherapeutic agent which was developed

as a result of observing the functional impact of perturbations in nuclear architecture on gene expression.

The modulation of chromatin structure within the nucleus plays an important role in the regulation of gene expression. These modifications are carried out by histone acetyltransferases (HATs) and histone deacetylases (HDACs), which control the acetylation status of core histones on chromatin. The activation of transcription is associated with the acetylation of histones while repression is associated with deacetylation. HDAC inhibitors have been developed with a variety of effects in tumor cells, ranging from inhibition of cell proliferation, induction of differentiation, and induction of apoptosis [Vigushin and Coombes, 2002]. HDAC inhibitors have recently been approved for the treatment of cutaneous T-cell lymphoma, but they have the potential to be useful in many different cancers due to their effects on multiple pathways. Further, they may also be useful in combination with other established chemotherapeutic drugs. For example, in acute PML that has developed resistance to retinoic acid, treatment with the HDAC inhibitor Trichostatin A can restore the sensitivity of leukemic cells to retinoic acid [Grignani et al., 1998; Lin et al., 1998]. Similarly, the HDAC inhibitor valproic acid was shown to enhance sensitivity to retinoic acid in acute myeloblastic leukemia by modulating the expression of genes important in differentiation, induction of apoptosis and regulation of cell cycle [Trus et al., 2005]. In particular, the induction of p21 in response to treatment with the HDAC inhibitor valproic acid was interesting because it suggests that response to HDAC inhibitors could be monitored by examining the expression of p21.

The positioning of genes within chromatin in the nucleus has mostly been observed using DNA FISH techniques labeling the gene locus. However, these kinds of studies do not allow for the direct visualization of transcriptionally active gene loci. By using RNA FISH, we can examine the transcriptionally active subpopulation of alleles. In DNA FISH experiments, two gene loci may be visualized as being close together but these may not be the active alleles. Probes for RNA FISH bind nascent transcripts at the site of transcription and thus provide more precise localization data on transcriptionally active genes that are positioned within close spatial proximity. Such genes that are on in the same cells and in the same subregion of the nucleus may be coregulated. It is important to examine additional genes to clarify the relationship between spatial proximity and the regulation of gene expression and how this is perturbed in cancer cells.

THE SPATIAL POSITIONING OF TRANSCRIPTION SITES

Transcription is the main regulatory step in the control of gene expression and the nuclear organization of the transcriptional machinery has important consequences for tumorigenesis. There are two theories for how transcription is organized within the nucleus. One possibility is that active genes move to preassembled sites of transcription or "transcription factories" and the other more traditional view is that active genes recruit RNA Pol II and assemble transcription complexes. Evidence for the spatial clustering of transcribing genes comes from a study that examined the transcriptional activation and localization of multiple genes located on the same chromosome in mouse erythroid progenitors [Osborne et al., 2004]. Using RNA FISH, active genes were observed to colocalize in a transcription-dependent manner with the same RNA Pol II complex while identical non-transcribed alleles did not colocalize. Therefore, the authors concluded that active genes move to preassembled transcription sites instead of recruiting and assembling their own transcriptional machinery. Another study found that the human α and β globin genes, which are on separate chromosomes frequently associate when transcribing [Brown et al., 2006]. However, these studies do not rule out the possibility that genes may recruit RNA Pol II and then move into transcriptionally active regions of the nucleus. Furthermore, from these studies, it is not possible to conclude that these genes are sharing a transcription factory, since the distance separating the associated genes was observed to be greater than the reported size of transcription factories [Brown et al., 2006]. Examining the transcriptional activity of additional genes in different cell lines would help to clarify these issues. Furthermore, local variations in the availability of transcription factors may also play a role in regulating which genes are transcribed at a particular time. The

accumulation of transcription factors in a particular compartment of the nucleus may cause genes that are regulated by these factors to cluster within that region.

Analysis of the transcriptional activation of several genes simultaneously within individual nuclei by FISH for nascent mRNA has shown that genes cycle on and off and that many genes experience long periods of transcriptional inactivity so that each cell expresses a unique profile of genes at any given time [Levsky et al., 2002]. This indicates that individual cells within a population have a unique subset of actively transcribed genes and therefore may differ in their nuclear microenvironment and their response to physiological stimuli. Similarly, an analysis of individual cells within a population of cells from a tumor sample would be expected to identify particular subsets of cells which have important phenotypes such as metastatic potential or resistance or sensitivity to chemotherapy.

Imaging the transcriptional activation of individual genes in live cells has shown that genes pulse on and off at irregular intervals and revealed the presence of transcriptional memory which means that cells are more likely to reexpress a given gene than to activate new expression [Chubb et al., 2006]. The opportunity to examine living cells as they activate or repress gene expression in response to a specific environmental stimulus such as a chemotherapeutic agent will provide significant insight into the role of the nuclear microenvironment in the regulation of transcription.

In addition, recent advances in the application of FISH to tissue samples has enabled the correlation of gene expression profiles with overall cellular morphology and provided the opportunity to examine the links between the nuclear architecture and the regulation of gene expression within intact tumor specimens [Capodieci et al., 2005]. An examination of the mechanisms underlying alterations in nuclear structure observed in prostate cancer showed that the transcriptional coactivator p300 directly modulates nuclear morphology [Debes et al., 2005]. Previously, p300 was shown to have a direct role in the proliferation of prostate cancer cells and its expression was correlated with cancer progression [Debes et al., 2002, 2003]. These studies directly link changes in nuclear architecture with cancer progression and thus, markers with a role in transcription such as p300 may be useful in diagnostic pathology to define new subtypes of prostate cancer, to monitor tumor progression, and possibly response to treatment.

CONCLUSIONS

Much of the current experimental evidence seems to suggest a model of nuclear organization in which nuclear architecture is generated by self organization [Misteli, 2007]. In this model, the activation of a gene at a particular location in the nucleus causes the remodeling of chromatin, the recruitment of transcription factors and the activation of other genes in close spatial proximity. The precise organization of nuclear components suggests that the appropriate structures and factors must be present in the correct spatial location and at the right time for proper gene expression. This suggests that modulation of nuclear architecture has an important role in regulating gene expression and that the structural perturbations observed in cancer play an important role in mechanisms of tumor development and progression.

More detailed analysis of the complexities of nuclear organization and gene expression will require new experimental approaches that preserve the architecture of cellular components. Advances in the field of imaging and microscopy will yield new insights into the nuclear organization of gene regulatory machinery and identify new options for cancer detection and treatment. The development of nuclear-structure based assays for cancer diagnosis will allow potential chemotherapeutic drugs to be chosen according to whether or not they can reverse tumor-specific nuclear changes back to a normal cellular phenotype.

FISH is currently used in the diagnosis of many hematologic malignancies. Applying FISH to solid tumor samples allows for gene-expression profiling of individual tumor cells within intact tissue samples while preserving tissue heterogeneity. This technique would allow pathologists to combine traditional nuclear morphology-based diagnosis with molecular analysis of the transcriptional status of key genes. Further, a large number of cells could be evaluated in a high-throughput manner making FISH an important molecular tool for the detection of tumor cells with unique phenotypes such as sensitivity or resistance to a particular treatment or metastatic potential.

FISH for nascent mRNA can be combined with immunofluorescence to correlate gene expression profiles with markers of cellular proliferation or apoptosis.

By further analyzing the cancer-induced nuclear changes in tumor samples and the effects of treatments on nuclear function, we can gain new insight into the link between nuclear structure and the regulation of gene expression in cancer. The application of new molecular techniques to cancer diagnosis and treatment is expected to yield many insights into targeted therapies for solid tumors, which are now largely treated by non-selective killing of all rapidly proliferating cells.

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