Meeting report

Intranuclear changes in cancer cells

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A report on the FASEB meeting 'Nuclear Structure and Cancer', Saxtons River, USA, 16-21 June 2007.

This year's Federation of American Societies for Experimental Biology (FASEB) conference on nuclear structure and cancer opened with keynote speaker Donald Coffey (Johns Hopkins University, Baltimore, USA), who together with Ronald Berezney (University at Buffalo, Buffalo, USA) is credited with the discovery of the nuclear matrix. Coffey pointed out that changes in nuclear structure are diagnostic hallmarks of many cancers that are frequently utilized by the pathologist but are poorly understood from a molecular standpoint. This set the tone for a stimulating meeting, where talks on the fundamental organization of the nucleus were intermingled with reports of direct links to cancer and perspectives from pathologists. There were many interesting talks and here we report a few of the highlights.

Chromosomal organization and aberrations

How universal are the 'rules' of chromosome organization within the nucleus that are so frequently abrogated in cancer cells? Thomas Cremer (Ludwig-Maximilians-University, Munich, Germany) reported that in rod cells of the retina of nocturnal animals, the heterochromatin is in the center of the nucleus and euchromatin at the periphery, which is the inverse of nearly all other cell types examined to date. He speculates that this arrangement may be less diffractive to light and might, therefore, provide an advantage for nocturnal animals.

Live-cell imaging is now a standard tool in the analysis of nuclear structure and function. Andrew Belmont (University of Illinois, Urbana, USA) addressed the large-scale chromatin organization of actively transcribing genes in somatic diploid nuclei of living cells. In contrast to models proposing the transition from loops of 30-nm chromatin fibers to 10-nm fibers during transcriptional activation, Belmont finds that transcribed chromatin remains several-fold more compact than even the 30-nm fiber. How do transcription factors find their cognate sites in these thick fibers? Gordon Hager (National Cancer Institute, Bethesda, USA) emphasized the highly dynamic nature of transcription factor binding as illustrated by the implication of chromatin remodeling as a key mechanism in nuclear receptor 'hit-and-run'. He described recent work addressing the question of whether chromatin remodeling is always required for interactions of the glucocorticoid receptor (GR) with its binding sites and whether these are always dependent on Brg1, a component of the chromatin-remodeling SWI/SNF complex. Genome-wide analyses revealed that chromatin remodeling takes place at all GR-binding sites, but is not always dependent on Brg1. The results suggest that GR searches chromatin randomly, but that chromatin remodeling distinguishes functional and non-functional interactions. Hager also pointed out that mapping of DNase I hypersensitive sites is an important tool in the identification of functional regulatory sites where chromatin remodeling takes place.

A common characteristic of tumor cells is genomic instability leading to chromosomal aberrations. For example, aggregates of telomeres are frequently observed in the nuclei of tumor cells but not in those of normal cells. Sabine Mai (University of Manitoba, Winnipeg, Canada) reported that expression of the proto-oncogene *c-Myc* leads to the formation of telomeric aggregates and telomeric fusions. She has found that the normal intranuclear positioning of centromeres and chromosomes was disturbed in cells overexpressing c-Myc, and that this was associated with the formation of chromosomal aberrations. These nuclear changes required the Myc box II domain of c-Myc, which associates with the histone acetyltransferase (HAT) Tip60; c-Myc overexpression in combination with Tip60 heterozygosity led to enhanced lymphoma formation in

mice. These findings suggest that c-Myc-induced changes in nuclear organization play an important role in the genomic instability associated with tumorigenesis.

Jeffrey Salisbury (Mayo Clinic, Rochester, USA) presented an elegant study on the regulation of centrosome duplication. Centrosome amplification is often observed in tumor cells and can lead to aberrant spindle formation and chromosomal instability. Normally, centrosome duplication is coordinated with the cell cycle. During the cell-cycle arrest that follows DNA damage the protein xeroderma pigmentosum C (XPC) becomes upregulated. XPC binds to centrin 2, which is essential for centrosome duplication, and the complex moves into the nucleus - a movement dependent on the presence of the tumor suppressor protein p53. This depletes the cytoplasmic pool of centrin 2, and the resulting low levels are not sufficient to support centrosome duplication. Salisbury has found that in cells, such as tumor cells, that are defective in p53 function, or following post-transcriptional silencing of XPC, the cytoplasmic pool of centrin 2 remains high enough to support aberrant duplication, resulting in spindle abnormalities and chromosome instability.

The relation between cell-cycle stage and vulnerability to carcinogens was addressed by David Kaufman (University of North Carolina, Chapel Hill, USA), who has shown previously that cells are most susceptible to transformation by chemical carcinogens when treated at the beginning of S phase. He now reported that in two different human cell types (normal fibroblasts and lymphoblastoid cells), genes replicated at the beginning of S phase include the majority of apoptosis genes and Wnt genes, both of which are frequently associated with cancer. This suggests the existence of a class of important cancer-related genes, located on several different chromosomes, which are replicated simultaneously during the cell cycle and are most vulnerable to cancer-causing mutations during replication.

The organization of the sites at which DNA replication and transcription take place within the nucleus and their potential roles in the generation of chromosomal aberrations associated with cancer were addressed in several presentations. James Davie (University of Manitoba, Winnipeg, Canada) outlined work showing that transcription factors Sp1 and Sp3, which are ubiquitously expressed in mammalian cells, form distinct unrelated nuclear foci and associate with histone deacetylase (HDAC) 1 and phosphorylated HDAC2. Using chromatin immunoprecipitation (ChIP), he studied the binding of the estrogen receptor alpha, Sp1 and Sp3 to the estrogen-responsive trefoil factor 1 promoter in MCF-7 breast cancer cells. The findings suggested that Sp1 and Sp3 aid in recruitment of HDACs and HATs. Spectral karyotype analyses revealed high chromosomal instability in MCF-7 cells and Davie pointed out that the question of cell-to-cell variation in the karyotype of cancer cell lines must be carefully addressed when performing ChIP analyses.

The importance of the organization of the transcriptional regulatory machinery in nuclear microenvironments in cancer was underlined by work presented by Gary Stein (University of Massachusetts, Worcester, USA). He reported that the NMTS domain of the Runx transcription factors is necessary and sufficient for subnuclear targeting and trafficking. Perturbations in subnuclear targeting of Runx1 (also known as AML1) are associated with altered competence for myeloid differentiation and with expression of the transformed/leukemia phenotype in myeloid progenitors. Impaired intranuclear trafficking of Runx2 (also known as AML3) in metastatic breast cancer cells interferes with the formation of osteolytic lesions *in vivo*.

Cameron Osborne (Babraham Institute, Cambridge, UK) reported that immediate early genes, including *c-Myc*, are recruited within minutes into transcription factories - complexes of transcribing genes - upon B-cell stimulation. He has found that 25% of transcribing *c-Myc* alleles colocalize with the *IgH* locus. Other active loci did not show a correspondingly high degree of association. Intriguingly, *c-Myc* and *IgH* are the most frequent translocation partners in mouse plasmacytoma and Burkitt's lymphoma, suggesting that intermingling of chromatin from the *c-Myc* and *IgH* loci during co-transcription makes it more likely that these loci will undergo translocations.

Epigenetic changes in cancer cells

In addition to genetic lesions, cancer cells display characteristic epigenetic changes associated with altered gene-expression patterns, which include CpG island hyper- and hypomethylation. CpG island hypermethylation leads to epigenetic silencing by recruiting methyl-CpG-binding domain (MBD) family proteins. William Nelson (Sidney Kimmel Comprehensive Cancer Center, Baltimore, USA) reported that hypermethylation leads to epigenetic silencing of the *GSTP1* locus (encoding the π-class glutathione S-transferase) in nearly all prostate cancers, and most breast and liver cancers. High-throughput chemical screening has yielded a number of compounds capable of activating transcription from hypermethylated *GSTP1* promoters, and several of these compounds antagonized the binding of MBDs to methyl-CpG-containing DNA. Nelson described a strategy for characterizing methyl-CpG patterns based on the binding of DNA to the DNA-binding domain of MBD2 and subsequent hybridization to whole-genome tiled arrays. This approach has potential applications in cancer screening, detection, diagnosis and prognosis.

A direct link between oncogene activation and diagnostic nuclear changes in human papillary thyroid carcinoma cells was reported by Andrew Fischer (University of Massachusetts,

Worcester, USA). Changes diagnostic of this type of cancer are the dispersal of heterochromatin and irregularity of the nuclear lamina. Activation of either of the tyrosine kinases Ret or Trk is sufficient to induce the diagnostic changes *in vitro*. Fischer reported that dispersal of heterochromatin is associated with a decrease in monomethylation of lysine 4 of histone H3, and that Trk is sufficient to induce this decrease.

Previous work from the laboratory of Donald Tindall (Mayo Clinic, Rochester, USA) showed that the transcriptional co-activator p300 is involved in the progression of prostate cancer. At the meeting, Tindall reported that transfection of p300 into prostate cancer cells induces quantifiable nuclear alterations associated with prostate cancer. Furthermore, he proposed the provocative idea that androgen ablation, a routine treatment for prostate cancer, might initiate an androgen-independent cycle of activity. This suggestion is based on the findings that p300 mediates androgen-independent activation of the androgen receptor and that androgens downregulate p300 in cancer cell lines. Thus, androgen ablation might increase p300 expression in prostate cancer cells, which might initiate aberrant androgen-independent activation of the androgen receptor. Androgen-independent activation of the androgen receptor is usually observed in refractory prostate cancer after androgen-deprivation therapy.

The molecular basis by which alterations in nuclear structure and function lead to cancer is unquestionably an exciting new area for young researchers. Exchange between clinicians and basic researchers in this field is still limited, but is an inevitable prerequisite for investigating and understanding nuclear changes in cancer. This biennial meeting will continue to provide an exceptional forum for such discussions between clinicians, pathologists and basic researchers. The conference also provided a forum for investigators from academia and the biotech and pharmaceutical industries to explore cancer-related changes in the organization and localization of regulatory machinery within the cell nucleus as a platform for new diagnostic and therapeutic strategies.