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MEETING REPORT

Meeting Report on the 12th International Congress of Histochemistry and Cytochemistry (ICHC)

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The International Congress of Histochemistry and Cytochemistry (ICHC) promoted in San Diego La Jolla (CA, USA), the 12th meeting where researchers of all over the world presented their work and the most innovative methods in histochemical disciplines. A summary of the last meeting is reported. J. Cell. Physiol. 204: 407–411, 2005. © 2005 Wiley-Liss, Inc.

The 12th International Congress of Histochemistry and Cytochemistry (ICHC) was held on July 24–28, 2004 in San Diego La Jolla, California. As the President of International Federation of Societies for Histochemistry and Cytochemistry (IFSHC), Ron Van Noorden rightly thinks, this discipline remains very much alive and contributes significantly to our understanding of structure and function of cells and tissues. Moreover, most histochemical techniques are now extended to whole organs and organisms in order to study cell biological and pathological processes. In consideration of most significant developments in this discipline, the 12th ICHC was entitled "Cellular and Molecular Interactions in Development and Disease." In the last years, histochemistry and cytochemistry have particularly developed some strategies to: (1) increase sensitivity of in situ hybridization and immunohistochemical techniques at both the light and electron microscopic levels in order to detect low amounts of mRNA or protein in cells and tissues; (2) develop technologies based on green fluorescent protein (GFP) and related fluorescent proteins in combination with highly sensitive digital imaging in order to monitor tumor development in live animals; (3) generate 3-D images of live cells in multidimensional light microscopy; (4) improve EM tomography technology to allow 3-D reconstructions of a high spatial resolution of subcellular structures. Based on these new frontiers of histochemistry, the 12th ICHC selected several topics divided in thirteen symposia, nine plenary lectures and six workshops. In the opening lecture entitled: "Breeding Molecules to Spy on Cells," R.Y. Tsien (Howard Hughes Medical Institute, USA) presented an interesting new mechanism based on uptake of a wide variety of contrast agents into cells and tissues that are offering the possibility to specifically concentrate these agents, such as radioactive, magnetic, and infrared agents, in diseased tissues (Tsien, 2005). In addition, R.Y. Tsien received the David Glick Award in recognition of outstanding scientific contributions to the advancement and practice of Histochemistry and Cytochemistry. During the opening ceremony an other important prize, the Paul Nakane Prize in recognition of outstanding scientific contributions and international leadership in the advancement of the disciplines of Histochemistry and Cytochemistry was given to P.K. Nakane. In the first plenary lecture, entitled: "Calcified Tissues: The Backbone of the Life," A. Nanci (Université de Montreal, Canada) presented an overview on bones and teeth studies with particular attention on the creation of 'intelligent biomaterials' and of a 'surgical window' system to experimentally manipulate the cellular and matrix events implicated in the formation of calcified tissues (de Oliveira et al., 2003; de Oliveira and Nanci, 2004). In the Symposium: "In Situ Visualization and Manipulation of Functional Molecules" M.D. Cahalan (USA) explained a new model to visualize the cellular dynamics of T lymphocytes interacting with antigen-presenting dendritic cells within the lymph node during the initiation of an immune response (Miller et al., 2004); K. Fujita (Japan) presented techniques for observation of living cells and tissues using two-photon fluorescence (TPF) and second-harmonic generation (SHG); D.W. Piston (USA) explained the methods built from him to delineate NAD(P)H signals from the cytoplasm and mitochondria showing differences between glucose and pyruvate metabolism (Rocheleau et al., 2004); and finally T. Takamatsu (Japan) presented the biomedical applications of the near-infrared (NIR) laser-induced microdisruption focusing on generation of calcium waves in living cells and selective attenuation of connexin43 gap junctions (Suzuki et al., 2003; Tsujii et al., 2003). In the first Workshop entitled: "The Impact of Microscope-Based Technology on Problems in Diagnostic Pathology," C.M. van der Loos (The Netherlands) showed immunoenzyme double staining for co-localization studies, methodologies, and chromogens; R. Levenson (USA) described the application of multispectral imaging to multiplexing for both fluorescent and non-fluorescent imaging techniques; and S.M. Hewitt (USA) presented methods of data analysis in the interpretation of tissue microarrays and clinical trials of molecularly targeted drugs. In the concurrent Workshop entitled: "Advances in Correlative Microscopy and Cellular Imaging: Labels to Dye for," E. Rosa-Molinar (USA) reviewed his efforts to develop and characterize multi-functional probes and a novel multi-functional dendrimer correlative probe (MuD) for intravital

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imaging and correlative microscopy to visualize and manipulate retinoic acid synthesizing cells in the spinal cord; J.M. Rivera-Ortiz (USA) presented the synthetic methodology and the characterization of a small library of peptide-based dendrimeric multifunctional probes with also preliminary correlative microscopy studies on mosquitofish; and R. Albrecth (USA) showed labeling methods for high resolution correlative microscopy in particular focusing on the synthesis of uniformly sized colloidal metal particles of differing geometry and on the synthesis of uniformly sized colloidal metal particles of differing elemental composition. In the Symposium: "Development and Regeneration in the Nervous System," H. Li (China) showed the age-dependant change of huntingtin associated protein 1 (HAP1) expressing levels in the hypothalamus and neurohypophysis and the role of HAP1 in development of nervous system and in regulation of endocrinal function (Li et al., 2003); X. Gu (China) presented the effects of Nerve Regeneration Factor (NRF) on cultured rat brain cells and the signal transduction in PC12 cells showing that NRF could protect PC12 cells in serum-free medium, and it may function via ERK1/2 phosphorylation cascade potentially; and W. Cai (China) presented the expression of developmental related genes: *Bcl-2*, *Nov*, *Aromatase*, *Noggin*, and *BMP-4* in the brain and their regulation effects to the nerve cell differentiation. In the Symposium: "Discovery and Development of Tissue-Based Biomarkers in Prostate Cancer," B.S. Knudsen (USA) introduced this topic based on novel technological advances of analysis of genomic and proteomic expression in prostate tumors (Chen et al., 2004; Yu et al., 2004); P. Nelson (USA) focused on the fact that molecular correlates of pathological and clinical observations may provide resources for studies designed to determine mechanisms of cancer behavior; A.M. DeMarzo (USA) showed that the earliest phase of human prostate carcinogenesis may proceed as a consequence of chromosomal instability mediated by shortened, dysfunctional telomeres (Lapointe et al., 2004); M. Rubin (USA) provided an approach to validation of candidate genes to use as biomarkers for prostate cancer progression using tissue microarrays (Gaston et al., 2005); and C.C. Collins (USA) showed the use of array Comparative Genomic Hybridation (aCGH) to analyze a cohort of patients indicating that aCGH profiles revealed numerous recurrent copy number aberrations associated to different stages of this cancer. In the second plenary lecture entitled: "Rainbow Imaging In Vivo," R.H. Hoffman (AntiCancer, Inc., USA) presented a new cell biology where the behavior of cells can be visualized in the living animal using a dual-color fluorescence imaging obtained by red fluorescent protein (RFP)-expressing tumors transplanted in GFP-expressing transgenic mice. Multiple-color labeling of cells will enable multiple events to be simultaneously visualized in vivo including gene expression, ion fluxes, protein and organelle trafficking, chromosome dynamics and numerous other processes. In the third plenary lecture entitled: "Vascular Zipcodes in tumor angiogenesis and metastasis," E. Ruoslahti (The Burnham Institute, USA) presented tissue-specific and tumor-specific vascular markers using in vivo screening of phage-displayed peptide libraries to identify peptides that target the vasculature of individual tissues or tumors (Ruoslahti, 2004). In the Symposium: "Angiogenesis and Vasculogenesis," V.L. Bautch (USA) showed that vascular endothelial growth factor (VEGF) signaling is important in numerous processes necessary to form a proper vessel, including aspects of sprouting and network expansion, and in vessel patterning in time and space (Kearney et al., 2004); J.R. Hove (USA) showed that the physiology and development of the embryonic heart is linked by hemodynamics suggesting the importance of examining the interplay between genetics and epigenetic factors in analyzing pathogenesis of embryonic vascular defects and cardiovascular disease (Hove et al., 2003); and D.R. Abrahamson (USA) presented data about Hypoxia-inducible factors (HIFs) expression in developing mouse kidney indicating that its distribution may be critical for cell-selective expression of HIF targets that coordinate vessel development and kidney organogenesis (Freeburg and Abrahamson, 2004). In the third Workshop entitled: "Automated Histochemistry," C.-M. Chuong (USA) presented experiences of automated immunostaining and in situ hybridization from a research oriented, university based laboratory; T. Warford (United Kingdom) showed high throughput protein expression profiling using phage display antibodies, tissue microarrays, automated immunohistochemistry, image capture and analysis. In the 4th plenary lecture entitled: "The Discovery of Aequorin and GFP," O. Shimomura (The Photoprotein Laboratory, USA) presented his work focused on extraction and properties of aequorin and GFP and the use of these two fluorescent proteins as important research tools (Shimomura, 2005). In addition, he received the Pearse Prize to honor the contribution made to histochemistry by Professor A.G.E. Everson-Pearse. In the Symposium: "Genome Organization: Basic Principles and Clinical Implications," M.O. Olson (USA) showed the behavior of several nucleolar proteins during the cell cycle by a variety of techniques indicating that fluorescence recovery after photo bleaching (FRAP) studies revealed the importance of C-terminal nucleic acid binding region of nucleolar proteins. In the Symposium: "Muscle Cell Differentiation," J.R. Feramisco (USA) presented an overview of 3-D renderings of multi-component fluorescence image data sets of cells and tissues; A. De Luca (Italy) showed the functional significance of regulatory factors of cell cycle as cyclin T2a and PKN-alpha in muscle differentiation (De Luca et al., 2003); P.L. Puri (USA) showed that skeletal myogenesis can be separable in discrete steps with the contribution of distinct cytoplasmic cascade that can be involved also in muscle regeneration (Simone et al., 2004); and K.C. Arden (USA) showed the PAX3-FOXO1 fusion gene influences muscle cell differentiation and may contribute to the tumorigenic phenotype in alveolar rhabdomyosarcoma (Arden, 2004). In the 4th Workshop entitled: "Quantum Dots as Novel Probes for Fluorescence Histochemistry," X. Wu (USA) presented the development of a technology to produce luminescent semiconductor nanocrystals, Qdot quantum dots demonstrating the excellent capability of Qdot conjugates for multicolor immunofluorescence microscopy; Y. Xiao (USA) showed that quantum dot conjugates improve probe photostability and brightness in fluorescence in situ hybridization (FISH) detection systems; K.A. Roth (USA) presented a protocol for combined tyramide signal amplification and quantum dot labelling in order to increase the sensitivity and phostostability of fluorescence-based immunohistochemistry; J. Itoh (Japan) presented applications of quantum dots for bio-imaging at light, 3-D and electron microscopic level; and M. Dahan (France) showed quantum dots technology to observe the motion of individual receptors in the membrane of live neurons. In the 5th plenary lecture

entitled: "Genes and Gene Transcripts Under the Microscope," A.K. Raap (Leiden University Medical Center, The Netherlands) overviewed some techniques to label nucleic acid sequences inside the cells, in particular the padlock probing may improve discrimination between perfectly matching and single nucleotide mismatching sequences. Moreover, he received the Piet Van Dujin Lectureship for outstanding contributions to the field of Histochemistry and Cytochemistry. In the 6th plenary lecture entitled: "Pathways Illuminated: Visualizing Signaling in Living Cells," A. Newton (University of California at San Diego, USA) presented the use of fluorescent reporters to visualize the activity of two key regulatory kinases: protein kinase C and Akt/protein kinase B (Kunkel et al., 2004). In the Symposium: "Mast Cell Development and Disease," C. Oliver (Brazil) showed that undifferentiated mast cells give rise to very immature mast cells which then migrate to peripheral sites; M.-C. Roque-Barreira (Brazil) presented the inflammatory activities of lectins and their ability to induce mast cell degranulation and neutrophil migration (Moreno et al., 2003); and A.M. Dvorak (USA) overviewed on ultrastrucutural architecture of human mast cells in their individual microenvironments and as they respond during disease. In the Symposium: "Extracellular Matrix and Inflammation," W. Parks (USA) showed that the matrix metalloproteinase matrilysin (MMP-7) is a key extracellular proteinase regulating fundamental processes of re-epithelialization and inflammation at mucosal surfaces (McGuire et al., 2003); L.E. Wrenshall (USA) showed that localization of IL-2 in spleen and thymus is critical for both normal immune function and tissue morphology (Wrenshall et al., 2003); T.N. Wight (USA) illustrated studies demonstrating that particular components of the extracellular matrix function as pro-inflammatory components in developing vascular disease (Farb et al., 2004); P. Noble (USA) showed that hyaluronan fragments produced during tissue inflammation have an important role in regulating the inflammatory response; A.K. Majors (USA) showed that interactions of immune cells with hyaluronan produced by mesenchymal cells may be important for modulating and perpetuating the inflammatory response and is a potential therapeutic target for patients with chronic inflammatory conditions (Hascall et al., 2004); and A.E. Proudfoot (Switzerland) discussed the ability of chemokines to inhibit the symptoms of inflammation in different animal models of inflammation (Johnson et al., 2004). In the Symposium: "Visualizing of Signal Transduction" A. Miyawaki (Japan) showed that propagation of protein kinase C signaling represents an underlying mechanism for global neuronal maturation following local astrocyte adhesion; G.D. Prestwich (USA) presented the monitoring of in vitro enzyme activity, spatiotemporal changes of intracellular lipid concentrations, and identification of lipid–protein interactions; T. Kobayashi (Japan) showed that Ras signal transduction requires rapid, cooperative formation of activated-Ras signaling complexes including a specific scaffolding protein like SUR-8 rather that random collision of signaling molecules (Murakoshi et al., 2004); and N. Saito (Japan) focused on PKC-delta targeting induced by ceramide in a single cell and PKC-gamma targeting induced by physiological stimulation in the neuronal network suggesting the importance of spatio-temporal activation of the specific PKC subtype in various cell responses (Kajimoto et al., 2004). In the 5th Workshop entitled: "Spectral Imaging," J. Beechem (USA) showed that spectral resolution other than has the ability to resolve more cellular targets, allows the very facile elimination of complicating fluorescence backgrounds, such as autofluorescence of paraffin embedded tissue sections; S. Tille (USA) overviewed the cutting-edge solutions provided for a wide range of biomedical applications; S. Sanford (USA) showed that loading cells with quantum dots together with multiphoton spectral imaging allows the possibility of following multiple targets simultaneously in the same animal; M.E. Dickinson (USA)focused on studying cell signaling during embryogenesis using dynamic laser scanning microscopy in conjunction with multiple fluorescent protein variants and vital dyes; and A. Miyawaki (Japan) presented a serendipitous cruise inside cells. In the 7th plenary lecture entitled: "5-D Imaging of Tumor and Immune Cell Migration and Communication," P. Friedl (Rudolf-Virchow Center for Experimental Biomedicine and Department of Dermatology, University od Wurzburg, Germany) described the 5-D semiquantitative fluorescence techniques that provides several insights in cell motility and cell–cell communication. So, time-resolved 3-D Histochemistry in live cell and tissue models support a 'dynamic pathology' approach to monitor molecular cell and tissue dynamics in health and disease (Friedl, 2004). In the 8th plenary lecture entitled: "Intracellular Ca^{2+} Signaling: Structure and Function of IP_3 Receptor and its Role in Cell Function," K. Mikoshiba (Japan) showed that IP_3 receptor is involved in fertilization and in neuronal plasticity and is essential for determination of dorsoventral axis formation (Bosanac et al., 2004). In the Symposium: "Is Histochemistry an omic?," G.C. Coulton (United Kingdom) proposed that Histochemistry can be considered as a part of new 'omic' sciences as well as genomics, proteomics, cytomics and that it belongs to byomics that is the integrated application of science into a coherent strategy for understanding biological complexity; G. Gannot (USA) focused on histomathematics that may facilitate multiplex protein expression analysis of tissue sections toward a better understanding of disease processes improving diagnostic and therapeutic markers (Tangrea et al., 2004); and M.N. Moore (United Kingdom) described an integrative methodology, the ecophysomics, that draws upon the use of physical and chemical data coupled with diagnostic and prognostic cellular biomarkers and bioindicators for the detection of 'distressed signals' and evaluation of 'health status' (Moore and Noble, 2004). In the Symposium: "Cell adhesion and Cancer," D. Cheresh (USA) showed that protecting the host vasculature from the vascular permeability-promoting effects of VEGF may be sufficient to prevent the extravasation of tumor cells (Weis et al., 2004); W. Stallcup (USA) presented the study of pericyte role in microvascular development using the NG2 proteoglycan as a marker for nascent pericytes revealing the association of these perivascular cells with the tips of growing endothelial sprouts in pathological vasculature (Ozerdem and Stallcup, 2004); and J.R. Couchman (United Kingdom) showed that perlecan, a ubiquitous proteoglycan of basement membranes, regulates tumor cell growth and promotes tumor formation and angiogenesis, but this appears to be multifactorial, and largely independent of heparin sulphate–VEGF interactions (Jiang et al., 2004). In the 6th Workshop entitled: "Retrieval of DNA, RNA and protein from archival tissues by AR based methods," C.R. Taylor (USA) overviewed the mechanism of the antigen retrieval (AR), studies of effectiveness and standardization; T.J. O'Leary (USA) showed that formaldehyde

crosslinks stabilize antigens against the denaturing effects of high temperature, and that the reversal of these cross-links is necessary for the restoration of immunoreactivity; K. Kakimoto and K. Miyajima (Japan) proposed that high-temperature heating might remove the masking of epitopes hidden due to the molecular configuration of the native molecule; T. Boenisch (USA) presented data on the routine use of AR for all formalin-fixed tissue antigens that is important to avoid the inconsistencies of staining known to emerge as a consequence of variable lengths of fixation times; B. Jasani (United Kingdom) focused on the importance of using a standardized and optimized antigen retrieval method with a stringent internal and external quality control in routine diagnostic and clinical trials settings; J.M. Robinson (USA) described the incorporation of a permeabilization/denaturation step using sodium dodecyl sulphate that improves immunolabeling for certain antigens in both cells and cryosections of tissues; M.L. Ingram (USA) showed that histoids, the 3-D constructs generated in the co-controlled conditions, are expected to have advantages over the conventional standards of tissue sections, tissue microarrays or cell lines alone for IHC or FISH/CISH procedures; and S.-R. Shi (USA) focused on determination of optimal chemical reagents to develop simpler, and more effective heat induced DNA retrieval technique on archival formalin-fixed, paraffin-embedded (FFPE) tissue sections. In the Symposium: "Visualizing Proteolysis," R. Van Noorden (The Netherlands) described the development of novel chromogenic and fluorogenic substrates to visualize activity of proteases in living cells and tissues; B.F. Sloane (USA) described a novel confocal assay for functional imaging of degradation by live cells of a quenched-fluorescent (DQ) basement membrane protein, type IV collagen, embedded in Matrigel (Sameni et al., 2003); and W.M. Frederiks (The Netherlands) showed that in situ zymography provides data that extend our understanding of the role of specific proteinases in various physiological and pathological conditions (Frederiks and Mook, 2004). In the Symposium: "New Aspects of Neuropeptides, Regulation and Signaling," R.E. Papka (USA) showed that neurons expressing estrogen receptors have the capacity to respond to estrogen during late pregnancy influencing the synthesis and release of neuropeptides which may play a critical role in cervical ripening and parturition (Mowa and Papka, 2004); P.E. Sawchenko (USA) summarized the results of experiments in which pharmacological or genetic manipulations of one or more of corticotrophin-releasin factor (CRF) family members have been used to ascertain their involvement in specific aspects of the CNS response to central peptide administration or various types of stress; and R. Elde (USA) presented rapid advances using histochemical methods that led to a profound change in our understanding of the regulation and biological mechanisms that underlie the function and role of opioid receptors, and by inference, receptors for many other neuropeptides (Stone et al., 2004). In the 9th plenary lecture entitled: "The Molecular Morphology of SARS", J. Gu (School of Basic Medical Sciences, China) showed that examinations of tissue samples from various organs of SARS patients died at late stage of the infection and white blood cells collected at early and mid stages of the disease demonstrated that SARS virus did not only injure the lungs but also infected multiple organs and cell types and so molecular morphology played a key role in understanding the pathogenesis of SARS that will guide future prevention, diagnosis, treatment, and vaccine development of this new disease. About 177 posters have been presented. During the congress were also given 20 IFSHC Young Histochemist Awards and 14 HCS Young Histochemist Awards. Complete abstract of oral and poster presentations are available at the web site http://www.ichc2004.org/.

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