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Meeting Report

DATELINE Boston

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Abbreviations: JNK, c-JunNH_2-terminal kinase; IGF, interleukin growth factor; IL-4, interleukin 4; TNF, tumor necrosis factor; HPV, human papilloma virus; E6AP, E6-associated protein; NAIP, neural apoptosis inhibitory protein; ICE, interleukin converting enzyme; MHC II, major histocompatibility II; BMT, bone marrow transplantation; CT, chemotherapy; M/E, mucositis and enteritis; ENF, enteral formulation; PDT, photodynamic therapy; PARP, poly (ADP) ribose polymerase; MP4, myelin basic protein and proteoplipid protein; PCD, programmed cell death; NSAIDs, non steroidal anti-inflammatory drugs; CNS, central nervous system

Following the key observations and categorization by Dr. John Kerr (Univ. Queensland, Australia) of apoptosis as a novel defining concept in cell biology, the notion of apoptosis remained embryonic for a number of years. It was not until August 1991 at the Banbury Center at Cold Spring Harbor Laboratory that the concept of apoptosis and all that it implied in cell biology was brought to some focus. At that time, myself and Dr. David Tomei (LXR, Biotechnology, Inc.) invited about 40 of the leading critics in cell biology and other fields to debate the concept of apoptosis and its potential applications to no less than a dozen fields of research. Dr. John Kerr was a key member of that meeting, and enlightened all of the attendees concerning his intentions and the potential for the concept of apoptosis.

Since that time, numerous meetings have been held and many discoveries have been made regarding the application of programmed cell death in many fields. Indeed, one might suggest that the concept of apoptosis, and its application to the many research fields, has had one of the greatest impacts in most recent years in cell biology, developmental biology, cell signal transduction, cancer, and potential therapeutics. The recent *IBC Conference* on apoptosis focused primarily in the area of therapeutics and the application of the concept of apoptosis in the mitigation of disease and mortality. Listed below is the meeting outline and the presenters' excerpts. Following is a summary of additional critical parts gleaned from this important meeting.

The title of the sessions and the major talks were as follows (chairs of each session are listed first):

I. The apoptotic signaling pathway

1. Investigations on the role of ceramide in apoptosis, G. Smith (Glaxo Wellcome Research and Development)

Several laboratories have implicated ceramide in apoptosis signaling. The data suggest that control of ceramide synthesis and metabolism could be useful in the treatment of diseases including cancer, ischemia, viral infections, inflammation and others.

2. Signaling by stress-activated MAP kinases during apoptosis, R. Davis (Univ. Massachusetts, Howard Hughes Medical Inst.)

The stress-activated group of MAP kinases include the c-Jun NH_2-terminal kinase (JNK) and the p38 MAP kinase. Exposure of cells to inflammatory cytokines or to environmental stress causes activation of the JNK and p38 signaling pathways. Recent studies have provided evidence for the mechanism of JNK/p38 activation and the relevance of these signaling pathways to apoptosis.

3. Modulation of cytokine-dependent survival by targeting the IGF-IR, R. O'Connor (Apoptosis Technology, Inc.)

The IGF-I receptor activated by its ligands IGF-I, IGF-II or insulin has been shown to elicit potent anti-apoptotic or survival activity in response to diverse stimuli. As a critical first step to elucidating the survival signaling pathway originating at the IGF-IR, site-directed mutagenesis was used to identify domains of the IGF-IR required for the survival function.

4. Regulation of Fas-mediated apoptosis in lymphocytes, T. Rothstein (Boston University)

The sensitivity to Fas-mediated apoptosis of B lymphocytes is regulated in a receptor-specific fashion. CD40 signaling induces Fas expression and produces susceptibility to Fas killing. In contrast, it was observed that engagement of either the antigen or IL-4 receptor induces a state of Fas-resistance and resistance to apoptosis.

5. Early events of the TNF signaling pathways, H.-B. Shu (Tularik, Inc.)

Several intracellular signal transducing proteins, including the death domain containing proteins TRADD and RIP, the TRAF domain containing proteins TRADD and RIP, the TRAF domain containing proteins TRAF2, and the cellular inhibitor of apoptosis protein cIAP1, are recruited to TNF receptor 1 in a ligand dependent manner. These were all shown to modulate apoptosis.

II. Apoptosis and disease

1. Clinical opportunities in aging and apoptosis, E. Wang (Lady Davis Inst.–Jewish Gen. Hosp., Canada)

Elimination of the apoptosis-resistance phenotype may provide a pragmatic means to delay or repress the chance for cancer information in older individuals. Studies of genes regulating pro- or anti-apoptosis may provide not only new means for effective diagnosis, but also prognostic means leading to early detection and cost effective treatment.

2. Apoptotic cell death in cardiovascular disease, S. Umansky (LXR Biotechnology Inc.)

In vivo and *in vitro* models have shown the involvement of cardiac cell apoptosis in heart damage, induced by ischemia/reperfusion and by other cytotoxic treatments. Reperfusion induced expression of CD95 (Fas/APO-1) and alternative splicing of CD95 mRNA in dog cardiac cells indicates the possible involvement of CD95 receptor in reperfusion-induced death of cardiac cells.

3. Antisense targeting of E6AP elevates p53 *in vivo*, M. Rolfe (Mitotix Inc.)

Invasive cervical cancer is highly correlated with the presence of high-risk human papiolloma virus (HPV) types 16 and 18. The viral E6 protein is thought to abrogate p53 function by stimulating its degradation via ubiquitin mediated proteolysis in a reaction requiring E6AP (E6-Associated Protein). Using antisense ologonucleotides targeting E6AP, a reduction of E6AP levels *in vivo* leads to increased levels of p53 and apoptosis expression.

4. Cerebral ischemia and IAPs, G. Robertson (Apopto Gen Inc.)

Neurons which express high levels of neuronal apoptosis inhibitory protein (NAIP), a member of the IAP family of anti-apoptotic proteins, are selectively resistant to damage. Moreover, experimental manipulations which elevated NAIP expression, rendered neurons more resistant to ischemic injury. These results suggest that increasing IAP expression may be a novel therapeutic strategy for the treatment of neurodegenerative disorders such as stroke.

5. ICE family proteases after ischemia and reperfusion in the central nervous system, L. Denner (Texas Biotechnology Corp.)

ICE family proteases have been implicated in apoptosis in a diversity of normal and pathological processes. Ischemia followed by reperfusion induces apoptotic cell death in many organs including the brain (stroke), heart, kidneys, and lungs. The results discussed were relative to the hypothesis that ischemia and reperfusion lead to rapid and extensive apoptosis, which is mediated by ICE family proteases.

6. Induction of apoptosis in autoreactive T cells with soluble MHC II: Peptide complexes, E. Spack (Anergen, Inc.)

Apoptosis plays an important role in both the establishment of self tolerance and, in some cases, dysfunction of the immune system, leading to autoimmunity. Clinical trials based on this approach are in progress for the treatment of multiple sclerosis, and pre-clinical research is examining applications in myasthenia gravis, diabetes, and rheumatoid arthritis.

III. Novel therapies/applications in apoptosis

1. Application of apoptosis concepts to mitigation of chemotherapy-related toxicity: A clinical outcome with surrogate and signal markers, F. Cope (Abbott Laboratories)

Life-threatening side effects occur in patients undergoing bone marrow transplantation (BMT) and chemotherapy (CT). Serious aspects of BMT and CT are the induction of mucositis and enteritis (M/E). The basis of M/E is the induction of apoptosis and the loss of normal luminal epithelial cells where M/E is a surrogate marker of apoptosis. Using an enteral formulation (ENF) capable of increasing luminal cell refractoriness to apoptosis as measured by M and the signal markers, effects were compared to standard clinical practice. Preliminary results indicate that patients using ENF had significantly improved outcomes, as measured by markers of apoptosis. Preliminary data also suggest that 12-month mortality may be decreased.

2. Anti-cancer therapy with a light-activated drug promotes apoptosis, D. Granville (QLT Photo Therapeutics, Inc.)

Photodynamic Therapy (PDT) is a cancer treatment comprised of the uptake of an inactive form of a light-sensitive drug by tumor cells. After the drug has accumulated within the target tissue, it is irradiated with a specific wavelength of visible light. Activation of the drug, typically a non-metallic porphyrin, causes the formation of reactive oxygen intermediates and ultimately the death of the cells.

Members of the ICE family of cysteine proteases are involved in the pathway, and poly (ADP) ribose polymerase (PARP) is cleaved within fifteen minutes of light treatment. Iodoacetamide completely blocked the PDT-induced cleavage of PARP. Multiple protease activation pathways appear to be involved in PDT-induced cell death.

3. Novel peptoid modulators of neuronal apoptosis, A. Davies (Panorama Research, Inc.)

Deregulation of the Bcl-2 oncogene underpins both the etiology and the chemoresistance of many significant tumors was developed. A high throughput screen was developed to evaluate specific, synthetic inhibitors of Bcl-2, using genetically engineered cell lines, whose survival of an apoptotic stimulus is contingent on the functionality of the Bcl-2 protein expressed within them.

4. Antigen-driven apoptosis *in vivo* as an immune therapy for T cell mediated diseases, J. Mueller (Alexion Pharmaceuticals, Inc.)

Activation-induced apoptosis of T cells plays an important role in the induction of peripheral T cell tolerance. A novel chimeric protein comprised of the myelin basic protein and proteolipid protein (MP4) was used to treat experimental encephalomyelitis and resulted in a dramatic clinical and pathological improvement of disease. These findings suggest that *in vivo*, several mechanisms contribute to the T cell hyporesponsiveness in tolerant mice.

5. Serine proteases, PCD and apoptosis in skin, M. Seiber (Johnson & Johnson CPWW)

Epidermal keratinocytes undergo spontaneous PCD. A serine protease activity, partially purified from the conditioned media, is sufficient to induce cell death in culture. The serine protease is regulated via the Bcl-2 pathway. The role of serine proteases in epidermal differentiation and apoptosis *in vivo* was discussed.

6. Cyclooxygenase- and p53-independent induction of apoptosis by FGN-1, a drug for treating pre-malignant lesions, G. Piazza (Cell Pathways, Inc.)

Non-steroidal anti-inflammatory drugs (NSAIDs) have been shown to be efficacious for treating pre-malignant lesions by a mechanism involving increased apoptosis of neoplastic cells. Cyclooxygenase inhibition may not be necessary for these effects since FGN-1, a NSAID derivative, which lacks cyclooxygenase inhibitory activity, induces apoptosis, and is efficacious in experimental models of cardinogenesis. Understanding the mechanism of action of FGN-1 may provide a new strategy for the development of more efficacious and less toxic cancer chemopreventive drugs.

IV. Gene families in apoptosis as therapeutic targets

1. ICE-like cysteine proteases and apoptosis, D. Nicholson (Merck Frosst Centre for Therapeutic Research)

Two key functions of ICE/CED-3-like proteases are to disable key homeostatic and repair processes, and to mediate the systematic structural disassembly of dying cells. Therapeutics that modulate the activity of these proteases are attractive for therapeutic intervention in several human diseases where inappropriate apoptosis is prominent.

2. A new family of apoptosis-related genes, H. Melkonyan (LXR Biotechnology, Inc.)

Using differential display, a new family of genes involved in cytoskeletal stabilization which regulates apoptosis was discovered.

3. The role of ICE and its homologues in mammalian apoptosis, D. Livingston (Vertex Pharmaceuticals, Inc.)

ICE, CPP32, and related cysteine proteases process protein substrates at aspartate-containing cleavage sites. Due to their overlapping substrate and inhibitor specificities, identifying the role of each protease in the apoptotic cascade has proven challenging. Roles for each new enzyme in apoptosis was presented.

4. Regulation of ICE/CED-3 family protease activation in apoptotic cells, L. Fritz (IDUN Pharmaceuticals, Inc.)

The cysteine proteases of the ICE/CED-3 family are critical effectors of apoptosis. Aspartate-directed protease activity is rapidly generated in lymphoma cells following an apoptotic stimulus. Bcl-2, acting at, or upstream of the protease CPP32, blocks its activation and the associated cell death in this system. The analysis of protease activation has now been extended to additional tumor cell lines and primary neuronal cells, and to a mode system of Bax-induced death in the fission yeast *S. Pombe*.

5. Structure of Bcl-X_L, an inhibitor of programmed cell death, S. Muchmore (Abbott Laboratories)

The structure of Bcl-X_L is reminiscent of the membrane translocation domains of bacterial toxins, and in particular, the colicins and diphtheria toxin. This similarity provides insight into the function of these molecules.

As with any concept in science, to the extent that science has any direction, such a direction must embody the application of acquired knowledge for promoting human wellness; neither the investigators in the field nor the field of apoptosis are excepted. It is in this spirit that this

conference was convened. Much to the delight of many, apoptosis has provided a new model for approaching heretofore seemingly insoluble clinical problems for numerous diseases. For example, our work at Abbott Laboratories has shown that one can substantially reduce dose-limiting chemotherapeutic side effects, where GI symptomology is the dose-limiting factor, without risk of reducing the therapeutic index of the drug. Indeed, such an approach has provided enhanced therapeutic indices for a number of key chemotherapeutic drugs. In order to measure the defined potential for such an approach, one necessarily needs to understand how cells respond to environmental stimuli such as chemotherapeutic agents, and how they transmit the signal within themselves and to other cells. The first phase of the IBC conference as it related to cell signal transduction and apoptosis, was a key stage-setting phase of this conference. In particular, overviews of protein kinase cascades and the role of ceramide, cytokines, Fas, phosphorylation, cell matrix proteins, and membrane transport were all established as key approaches in evaluating signaling pathways. Clearly, all such venues are involved in defining the tenacular transduction of both intracellular, transcellular, and organismal expression of apoptosis. The elucidation of new compounds to either enhance or inhibit apoptosis, where such compounds have a significant role in defining signal transduction pathway selection and shunting of signals was discussed. The application of these compounds to reduce survivability of malignant cells or enhance survivability of normal cells, was a key issue in evaluating potential therapeutic indices of a number of new drugs. Such drugs may be differentiated by their ability to act only as pro drugs in target cells.

A perennial focus in programmed cell death has been the expression and utility of bcl-2, BAX, and the expression/activation of proteases, including ICE and CED-3. Most recently a paper has been published suggesting that bcl-2 might act in a membrane transport system and the reconciliation of this with its perceived role as an anti-oxidation molecule remains to be accomplished. Below are some additional key focal points from the meeting:

I. Key areas of clinical investigation
- Apoptotic cell death in cardiovascular disease.
- Clinical potential in aging and apoptosis.
- Cerebral ischemia and CNS damage reduction.
- Chemotherapy and mitigation of malignant disease.
- Mitigation of autoimmune disease/promulgation of immune function.

II. Specific areas of research
- A significant reduction in reperfusion injury-induced cell death in cardiac cells has been achieved.
- A new family of apoptosis-related genes has been discovered that relate to cytoskeletal stabilization.
- Significant progress has been made in inhibiting autoimmune-induced apoptosis and the induction of tolerazation as a clinical approach for the reduction of neuropathology.
- Significantly new and efficient *in vitro* screening systems have been developed for the evaluation of inhibitors of bcl-2 as a therapeutic target.
- Substantial clinical progress has produced a clinically relevant reduction in GI toxicity induced by chemotherapy and radiation therapy for both bone marrow transplant and colorectal cancer patients.
- The application of numerous concepts as presented at this meeting have resulted in novel diagnostic approaches for presence of disease and disease evaluation.
- The use of non-steroidal anti-inflammatory drugs has provided an efficacious means for treating pre-malignant lesions by increasing apoptosis in neoplastic cells.

In summary, the *IBC Third Annual International Conference on Apoptosis and Its Application to Diagnosis and Therapy* provided an excellent venue for the exchange of information that may be critically important in the short term for the diagnosis of disease and for the immediate incorporation into small clinical trials. This is the necessary return on both intellectual and capital investment in a highly productive area of cell biology.