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VIEWPOINT

Meeting Report—UICC Study Group on Basic and Clinical Cancer Research: Apoptosis

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The purpose of these study group meetings, which are organized by the Tumor Biology Program of the International Union Against Cancer (UICC), is to establish a basis for possible clinical applications founded on molecular concepts. For this purpose, generally a few clinicians, pathologists, and epidemiologists are invited together with a core group of cell and molecular biologists. The meetings are of a particularly informal nature, to foster the exchange of ideas rather than to discuss data. It is for this reason that no book is published as a follow-up but rather the present brief report. More detailed data can be requested from the participants directly. Their addresses are provided at the end of this report.

Most meetings were devoted so far to oncogenes and tumor suppressor genes participating under normal conditions in the cell cycle and growth [Burger et al., 1988; Levine and Burger, 1993; Burger and Harlow, 1995]. One meeting, however, dealt already with the state of knowledge on programmed cell death 8 years ago [Burger and Harris, 1995]. Even though many of the components of the pathway in *Caenorhabditis elegans* had been defined at that meeting by Robert Horwitz, plenty of questions particularly on the regulatory input leading to apoptosis and on possible applications in medicine were widely open.

It was therefore tempting to revisit programmed cell death 8 years later and select with the help of Stan Korsmeyer and Bob Horvitz a few key players in the area to review the progress in this field. It is clear in the meantime that the apoptosis signaling chain in *Caenorhabditis elegans* has not only become essentially gapless and sophisticated but conceptually confirmed also in higher animals. It has also become clear that programmed cell death is not only a mechanism assuring proper embryogenesis but it also guarantees maintenance of a healthy state in adult cells as well for instance by weeding out cells with faulty DNA or with a faulty nutrient supply. The impressive increase in knowledge not only of apoptotic signaling but growth and metabolic signaling altogether has provided new interconnections not only at the signaling level but it has also brought insights at the level of biological functions.

This meeting did not only reveal such progress but also expanded into related processes to apoptosis like autophagy and necrosis and potential insights into pathogenic mechanisms of neurological diseases and of myocardial infarction where the control of apoptosis may contribute to potential therapies.

KINASE SURVIVAL PATHWAYS

Dr. Stanley Korsmeyer opened the meeting with an introduction on the gateways to apoptosis, which slowly grow in their complexity. In

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particular the Bcl-2 family related/interacting proteins that function as pro- or anti-apoptotic molecules. The expanded Bcl-2 gene family is defined by conserved α-helical domains, BH1-4, which regulate dimerization and function. Pro-apoptotic "BH3-only" members like BAD and BID interconnect with proximal death signals and trigger activating conformational changes of the pro-apoptotic "multidomain" members BAX, BAK. He then discussed his own results on the proteomic analysis of liver mitochondria. They revealed that the pro-apoptotic molecule BAD resides in a macromolecular complex with PKA and PPI catalytic units, Wiskott–Aldrich family member WAVE-1, and unexpectedly glucokinase. BAD is required to nucleate the complex and Bad-deficiency blunts mitochondrial glucokinase activity and thereby glucose induced respiration resulting in a diabetic phenotype. This BAD/glucokinase complex integrates major pathways of survival, glucose metabolism, and apoptosis.

Along this interrelationship between apoptosis and carbohydrate metabolism Dr. Craig Thompson hypothesized that cells from multicellular animals have lost the ability to take up nutrients in a cell autonomous manner as the single cell eukaryote yeast does. Metazoan cells depend on extracellular signals to stimulate the uptake of sufficient nutrients to maintain cell survival and anapleurosis. Bcl-2 proteins only regulate the length of time a cell survives in the absence of nutrients but it does not affect nutrient uptake. Two signaling pathways have been identified that regulate nutrient uptake and glycolysis: the PI3K/AKT/TOR pathway and a JAK/STAT/Pim pathway.

Genetic studies in flies and worms and cell biological studies in mammals indicate that the phosphoinositide 3-kinase (PI3K/AKT) pathway is a central mediator of cell growth, survival, and cell cycle entry. Dr. Lou Cantley reminded the participants that AKT phosphorylates a number of cell proteins and in most cases turns off the functions of these proteins. The key targets of AKT are themselves negative regulators of cell growth, survival, and cell cycle entry and thus, phosphorylation by AKT releases brakes on cellular responses. Recently, Dr. Cantley's group discovered that AKT phosphorylates the protein Tuberin and that this phosphorylation eliminates the ability of Tuberin to block activation of the mTOR protein kinase. This then provides a pathway by which activation of the PI3K/AKT pathway results in increased protein synthesis and cell growth.

CANCER AND APOPTOSIS

This topic prevailed in many presentations during this meeting but was particularly focused upon in the following three presentations.

Disruption of apoptosis can promote tumor development and resistance to cancer therapy, and precisely how apoptotic pathways are disrupted can produce treatment heterogeneity. Dr. Scott Low has shown that AKT and Bcl-2 can each cooperate with myc in lymphangiogenesis and produce chemoresistance. Interestingly, rapamycin can restore chemosensitivity to AKT-overexpressing tumors but not those overexpressing Bcl-2. These results support the view that knowledge of drug resistance mechanisms and tumor genotype can be used to effectively tailor cancer therapy.

It was pointed out by Dr. Arnold Levine that even though autophagy is a process different from apoptosis it would be unlikely if defects in autophagy might not also be involved in cancer. After starvation m-tor kinase activity drops, protein synthesis slows via the S6 kinase pathway, and vesicles which form containing cellular organelles fuse with lysosomes. The Beclin-1 gene product is an essential protein for the process of autophagy. *Beclin-1* gene knockouts in mice are lethal by 7.5–8.5 days after fertilization. Heterozygote mice with one wild type copy of Beclin-1 develop cancers (B-cell lymphomas, liver and lung cancers) by 15–20 months after birth. The tumors retain one copy of a wild type *Beclin-1* gene and tumor tissues have lower levels of Beclin-1 protein. This represents a new class of tumor suppressor genes where low levels of Beclin-1 may compromise autophagy, giving rise to tumors.

By inactivating tissue specifically pRb and related proteins via transgenic expression of a truncated SV40 T antigen (T121), Dr. Terry Van Dyke could show that similar biological responses (like aberrant proliferation and concomitant apoptosis predisposing to malingnancy in every case) were regulated by distinct pathways that depend on different cell types. For example, while pRb pathway disruption induces similar levels of proliferation and apoptosis in breast and prostate epithelium as well as astrocytes, apoptosis in the mammary gland is regulated by

p53 while it is regulated by PTEN in prostate and astrocytes. Such differences have significant implications for the mechanisms of tumor progression and the biological behaviors of respective tumor types. Indeed in brain epithelium p53 loss promotes both a reduction in apoptosis and progression to solid angiogenic tumors. In astrocytes, using a conditional PTEN allele, loss of PTEN causes a reduction in astrocyte apoptosis, an increase in astrocyte migration and invasiveness, and ultimately angiogenesis.

APOPTOSIS PATHWAYS

There are still plenty of unsolved questions both as to fundamental aspects of programmed cell death as well as to causes that trigger it or the fluxrates at the pathway steps.

Thus, Dr. Robert Horvitz reminded the participants that although a molecular genetic pathway for programmed cell death in *C. elegans* is well described, many aspects of the process remain to be elucidated. Key questions include: are there caspase-independent programmed cell deaths? How does phagocytosis promote cell killing? How do Rb, DP, and E2F proteins act in programmed cell death? What actually causes cells to die?

Dr. Marc Kirschner developed an in vitro system using the cytoplasm of frog eggs for studying the regulation of the canonical Wnt pathway downstream of Dsh and modeled it by a set of differential equations. He and his group had to cope with the exceedingly low concentration of axin, but they could show that increased concentrations of axin inhibit β-catenin degradation and that the cause for that is probably the formation of non functional complexes. Control coefficiences showed quantitatively the degree of oncogene activity and tumor suppressor activity.

The tumor suppressor BRCA-1 protein has been shown to have many functions and Dr. David Livingston reminded the participants that it is not well understood how it performs these functions. It is known, however, that when overexpressed it can elicit apoptosis. BRCA1 is a protein, dedicated in part, to DNA damage control. BRCA1 executes this function, in part, by directing certain DNA repair proteins to sites of DNA damage. At these sites, there are signs that heterochromatin superstructure is forming, and an interesting question is whether this phenomenon is also under BRCA1 control.

CELL DEATH IN DISEASES

In the forefront are ischemic, neurodegenerative and traumatic diseases primarily of the nervous and cardiovascular system.

Thus, axonal degeneration can occur by an active process in both normal development and in disease, especially in neurodegenerative diseases. Dr. Martin Raff pointed out that there is increasing evidence that this axonal self-destruct program depends on ubiquitination and proteasome degradation and has been conserved in evolution from flies to mice. He proposed that chronically insulted nerve cells may use this program to disconnect from their target cells—a process called “dying back”—to conserve energy in case conditions improve. This would explain why “dying back” is the most common pathology in neurodegenerative diseases.

Cardiac myocyte apoptosis occurs during ischemia-reperfusion injury and heart failure. Using a variety of genetic and pharmacological approaches, it was demonstrated by Dr. Richard Kitsis that cell death is an important component of these diseases. Thus, inhibition of apoptosis reduced infarct size in animal models of myocardial ischemia and lethality in heart failure models. Apoptosis may provide a target for novel therapies directed at these disorders.

A correlation exists between neuronal death in neurodegenerative disease and protein aggregation. Dr. Peter Lansbury proposes that pore-like protein aggregates may be the pathogenic entity in some of these diseases. Thus, a pore-like oligomer of a mutant form of superoxide dismutase that is linked to amyotrophic lateral sclerosis causes mitochondria to release cytochrome C.

Dr. Junying Yuan pointed out that increasing evidence suggests that neuronal cells may have multiple “programmed” mechanisms to die. Although apoptosis is very well characterized the alternative mechanisms of cell death are unknown. Two major proposed alternative mechanisms are autophagy cell death and necrosis. The evidence supporting a role of autophagy in cell death is not strong. It is possible that cells may activate autophagy as an autocremation mechanism. The studies on necrotic cell death in *C. elegans* suggest that necrosis is

mediated by specific forms of calpain and aspartyl proteases. A model was presented for a possible mechanism of mammalian cell necrosis.

INHIBITOR OF APOPTOSIS PROTEINS (IAP) AND INHIBITORS OF IAP (Smac)

Induction of apoptosis involves the coordinated action of proteins that promote ("gas") and inhibit ("brake") caspase activation. Dr. Hermann Steller discussed the concept that Reaper-like proteins remove the "brakes on death" by binding to and inhibiting IAPs. This includes the stimulation of IAP-autoubiquitination and degradation by Reaper. Insights into the underlying mechanism will allow the design of small molecules that may selectively kill certain cancer cells by eliminating IAPs that are frequently overexpressed in tumors.

Using structural biology, Dr. Yigong Shi and others have deciphered the precise molecular mechanisms of IAP-mediated inhibition of the initiator caspase, caspase-9, as well as the effector caspases, caspase-3 and -7. Although different sequence motifs of IAPs are used for these inhibitions, Smac/DIABLO uses the same scaffold and IAP-binding tetrapeptide motif to remove the inhibition. The intimate link between IAPs and cancer suggests a potential therapeutic application for Smac tetrapeptide-like molecules.

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APPENDIX A

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