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Meeting Report

Death in paradise

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Apoptosis and Cancer: Basic Mechanisms and Therapeutic Opportunities in the Post-Genomic Era
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Picture a starry sky, luminous waves, throbbing drums, the smouldering smell of roast pig, and a huddle of scientists discussing death. Not exactly paradise, at least for the pig, but that is what we experienced at this year's AACR Apoptosis and Cancer: Basic Mechanisms and Therapeutic Opportunities in the Post-Genomic Era meeting (organized by John C Reed and Scott W Lowe) on the Big Island of Hawaii. Aside from snorkelling, scuba diving and traipsing through volcanic craters, this year's speakers enthralled attendees with their recent discoveries in the field of apoptosis and cancer research. From the first night, the words of the former AACR president, Don Coffey, became chillingly clear, 'The only good cancer cell is a dead cancer cell'.

On the heels of the completion of the human genome project and taking a bioinformatics angle, John C Reed (The Burnham Institute, La Jolla, CA, USA) eloquently reviewed the complexity of human apoptosis-regulatory proteins. Michael Hengartner (University of Zurich, Zurich, Switzerland) then followed with an equally captivating discussion regarding apoptotic pathways in *C elegans*, and John M Abrams (UT Southwestern, Dallas, TX, USA) enlightened us regarding the *Drosophila* cell death field. This meeting explored far more territory than can be covered in this review, and unfortunately, some admirable research will be left out due to space constraints. Now, on to the talks.

In light of the resemblance between CHK-2 Li-Fraumeni syndrome and p53 Li-Fraumeni syndrome, Tak W Mak (University of Toronto and Ontario Cancer Institute, Toronto, Canada) demonstrated that p53/MDM-2 binding is inhibited by phosphorylation of Ser20 on p53 by CHK-2. Another role for p53 kinases was presented by Yoichi Taya (National Cancer Research Institute, Tokyo, Japan). Taya discussed how phosphorylation of p53 on Ser46 regulates its transcriptional activation of apoptosis-inducing genes, such as p53AIP1, and their search for the kinase responsible.

Autophagic death in response to steroid hormone treatment in *Drosophila* was addressed by Eric H Baehrecke (University of Maryland Biotechnology Institute, College Park, MD, USA). To identify genes with similar or differing expression patterns during steroid-triggered autophagy *versus* radiation-induced apoptosis, Baehrecke's group employed DNA microarrays as well as enhancer-trap genetic screens. Results showed that while steroid-triggered apoptosis involved large scale changes in gene transcription (hundreds of genes were induced), relatively few (less than thirty) genes were induced during radiation-induced apoptosis. Transcriptional changes were found for cell remodeling factors, proteolysis genes (including caspases) and DNA binding proteins.

In a system using primary lymphomas spontaneously generated in Eμ-myc mice expressing myc in the B-cell compartment, Scott W Lowe (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, USA) explored the role of p53 in drug resistance. Lymphomas with wild-type p53 were re-introduced into syngeneic mice and the mice were subjected to cytotoxic agents. As one might expect, tumors that maintained wild-type p53 responded well to treatment, tumors that lost p53 expression relapsed after initial responses, and tumors constitutively overexpressing Bcl-2 were non-responsive. Most importantly, Lowe showed that *p53*+/− tumors overexpressing Bcl-2 did not lose the second *p53* allele, clearly demonstrating no selective advantage for loss of *p53* in this instance, further stressing the anti-apoptotic function of p53 in tumor formation and progression. Along similar lines, using tumor cells engineered with inactivated p53 or Bcl-2 overexpression, Andrei V Gudkov (Cleveland Clinic Foundation, Cleveland, OH, USA) found that suppression of apoptosis by Bcl-2, in contrast to p53 inactivation alone, resulted in less metastasis *in vivo*. This suggests that inactivation of p53-dependent apoptosis via Bcl-2 overexpression is advantageous for tumor growth, as is p53 inactivation, but Bcl-2 overexpression does not affect metastasis nor genomic stability. He also introduced an elegant suppression selection approach (SSA) to sidestep the age-old problem of screening for genes that promote death. Gudkov added that this novel technique can be used to screen for temperature sensitive mutations and that he is open to collaborations.

Challenging the present-day dogma that numerous and diverse mutations are required for the establishment and maintenance of tumors, Gerard I Evan (UCSF Cancer Center, San Francisco, CA, USA) argued that tumorigenesis may require only a limited balance of mutations. Using an inducible mycER fusion construct expressed in pancreatic islet cells, Evan described the apoptosis of those cells after induction of the fusion protein with tamoxifen and

showed that co-expression of Bcl-x_L (to inhibit the pro-apoptotic function of myc) led to an outburst of β cell-adenoma formation. Evan's data narrows down the requirement for genetic aberrations to just two complementary events and strongly supports the crucial role of apoptosis suppression in cancer development.

Tak W Mak also stressed the importance of cell survival genes in tumor formation and progression. As tumors grow, they acquire mutations that promote uncontrolled growth and suppress death, but the majority of mutations observed actually affect cell survival, not apoptosis. In MALT (mucosa-associated lymphoid tissue lymphomas), the *Bcl-10* survival gene is truncated at its C-terminus resulting in constitutive NF-κB activity. It was reported that one-third of *Bcl-10*$-/-$ embryos die during embryogenesis due to a failure of neural tube closure and display elevated levels of apoptotic cells. Intriguingly, the remaining two-thirds survive, and their cells show no difference in sensitivity to cisplatin, suggesting that Bcl-10 is not involved in apoptosis.

On the death ligand and receptor front, there were many interesting talks with important ramifications in terms of cancer therapeutics. For example, although *Trail* mRNA is expressed in normal tissues, they appear to be TRAIL-resistant in contrast to tumor cells which are TRAIL-sensitive. This differential sensitivity to TRAIL may have important utility in cancer therapy strategies. Combinations of anti-cancer agents and/or genetic factors may affect the outcome of these TRAIL-based therapies. With this reasoning, Wafik S El-Deiry (University of Pennsylvania School of Medicine, Philadelpha, PA, USA) reported that TRAIL-induced cell death is significantly increased by concomitant c-*myc* or p53 expression/stabilization. Klaus-Michael Debatin (University Children's Hospital, Ulm, Germany) showed that betulinic acid can cooperate with TRAIL to induce apoptosis, suggesting that betulinic acid and its derivatives could be new promising drugs for the treatment of neuroectodermal tumors. (On a side note, Debatin also challenged the present day dual treatment of tumors with dexamethasone and cisplatin. In this system, dexamethasone antagonizes cisplatin-induced apoptosis of lung carcinoma cells by inhibiting caspase activity). In the same context, Ralph Schwall (Genentech, Inc., San Francisco, CA, USA) presented data where camptothecin pretreatment sensitized *Bax*$-/-$ cells to TRAIL, *in vivo*. Although these combinational strategies using TRAIL appear very promising, careful consideration of TRAIL's toxicity toward normal human hepatocytes will be necessary before using such treatments in a clinical setting.

One possible explanation for the differential sensitivity of normal *versus* tumor cells to TRAIL is the expression of c-$FLIP_L$. El-Deiry's results that c-$FLIP_L$ expression protects against TRAIL-induced death in HCT116 cells reflects c-$FLIP_L$'s actions as a 'competitive inhibitor' in precluding the binding of caspases to FADD in TRAIL-TRAIL receptor and CD95L-CD95 receptor pathways. Marcus E Peter (University of Chicago, Chicago, IL, USA) intrigued the audience by presenting c-$FLIP_L$ as both an inhibitor and an enhancer of CD95-mediated apoptosis. He reported that dimerization of c-$FLIP_L$ induces processing of caspase-8, thereby enhancing CD95 mediated apoptosis. Surprisingly, both the p18 and p10 subunits of c-$FLIP_L$ are required for this caspase-8 activation.

Increasing the complexity, Steven M Frisch (The Burnham Institute, La Jolla, CA, USA) reported that FADD is pre-associated with the CD95 receptor, but only in suspended cells. (FADD is a nuclear protein in attached MCF10a, HT1080 and HUVEC cells.) Frisch revealed that cell detachment induces FADD export into the cytoplasm. The function of FADD in the nucleus of epithelial cells remains unknown.

Joining the war against anthrax, Michael Karin (UCSD School of Medicine, La Jolla, CA, USA) described the role of anthrax lethal factor, a metalloprotease, in apoptosis and necrosis induction and its ability to cleave MAPKK.

On the hunt for survival signals induced by Raf activation, Julian Downward (UK Cancer Research, London) summarized the results of a gene chip analysis concerning transcriptional changes that occur in EGF-like growth factors in response to Raf activation. Providing a further link between growth factor signalling and cell death machinery, Hermann Steller (The Rockefeller University, New York, NY, USA) reported that Hid cell killing activity can be inhibited by MAPK phosphorylation and that the specificity of the EGFR/Ras/MAPK pathway seems to be solely for Hid, and not for Rpr nor Grim. Looking at survival signals during *Drosophila* CNS development, midline glial (MG) cells that fail to contact axons undergo Hid-dependent apoptosis, presumably due to a lack of phosphorylation by MAPK. The EGFR ligand, Spitz, promotes MG survival when presented by glial cells, setting up a scenario where MG cells compete for Spitz on axons, and in the absence of signaling, death ensues.

As mitochondria play a crucial role in the regulation and induction of apoptosis by their release of several pro-apoptotic proteins into the cytosol of cells, the ever-growing Bcl-2 family of proteins are being revealed to influence this step by interacting directly or indirectly with this organelle. Suzanne Cory (The Walter and Eliza Hall Institute for Medical Research, Melbourne, Australia) provided an overview of the family and presented a crucial role for Bim during thymocyte negative selection. Using *Bim*$-/-$ mice, it was shown that *Bim*$-/-$ thymocytes are resistant to TCR-induced apoptosis.

So, who still thinks that apoptotic mechanisms are simple? Apparently not Yuri Lazebnik (Cold Spring Harbor Laboratories, Cold Spring Harbor, NY, USA) who amusingly compared all the apoptotic signaling machineries with the electrical connections inside an old-style Russian radio. Using RNAi, Lazebnik showed that caspase-2 silencing inhibits apoptosis induced by etoposide, cisplatin and UV in IMR90E1A cells. He argued that caspase-2 is required for *cytochrome* c and smac release from the mitochondria and for Bax translocation to the mitochondria during etoposide-induced apoptosis. This observation is contrary to the conventional belief that this caspase acts downstream of caspase-3 in this pathway.

In terms of actual mechanisms, Yoshihide Tsujimoto (Osaka University Graduate School of Medicine, Osaka, Japan) discussed the regulation of mitochondrial outer

membrane permeability by Bcl-2 family proteins. Evidence concerning the interaction of Bcl-2 members with VDAC was proposed and Tsujimoto emphasized the anti-apoptotic activity of BH4 peptides. Craig B Thompson (University of Pennsylvania, Philadelphia, PA, USA) presented a comparison between cell survival induced by Bcl-x_L *versus* Akt. Akt-mediated survival is dependent upon promoting glycolysis and maintaining a physiological mitochondrial potential, while Bcl-x_L-mediated survival involves the preservation of mitochondrial integrity despite reduced mitochondrial membrane potential (resulting from the low glycolytic rate in growth factor-deprived cells). Thompson also presented striking Positron Emission Tomography (PET)-Scan images of patients using the glucose metabolism tracer, 2-deoxy-2-fluoro-D-glucose (FDG), to visualize the accumulation of the tracer in lung carcinomas (display up-regulated glycolysis), illustrating the importance of the glycolytic pathway in cancer.

Emad S Alnemri (Thomas Jefferson University, Philadelphia, PA, USA) provided new insight into the structure and the function of the serine protease OMI. OMI has a pyramid shaped homo-trimer structure with IAP-binding motifs at the top and PDZ-regions at the bottom. Via RNAi, a dual role for OMI was uncovered; while OMI binds to IAPs and promotes apoptosis, it also is involved in the maturation of mitochondrial proteins. The audience was further tantalized by the results of a screen for XIAP BIR3 binding proteins. In addition to fishing out SMAC and OMI, an 80 KDa mitochondrial protein was identified, but when asked, Alnemri remained tight-lipped.

Douglas R Green (La Jolla Institute for Allergy and Immunology, La Jolla, CA, USA) was scheduled to speak in the 'p53 family and apoptosis' session, however, in agreement with the chairperson (Green himself) he finally decided to present results concerning the mitochondrial pathway of death in *Drosophila*. Providing further insight into the cell biology of *Drosophila* cell death, Green concluded that although ARK (Apaf-1 homologue) plays a central role in stress-induced apoptosis in *Drosophila* cells, this apoptotic pathway seems to act independently of *cytochrome* c.

The results of a large-scale genetic screen for modifiers of the partial eye ablation phenotype that results from the ectopic expression of *reaper*, *hid* and *grim* in the developing retina of *Drosophila* were also relayed by Hermann Steller. Interestingly, lethal mutations of the Diap1 ring finger domain were observed that resulted in excessive embryonic apoptosis. Whereas Diap1 promotes the ubiquitinization of Dronc via its ring finger domain interaction with the E2 ubiquitin conjugating enzyme, ubcD1, ubcD1 mutant clones display increased levels of Diap1, suggesting that ubcD1 may promote Diap1 self-degradation. Steller proposed that in the absence of Rpr, caspase bound to Diap1 is ubiquitinated by ubcD1 and targeted for destruction, whereas in the presence of Rpr, the caspase is displaced, allowing for ubcD1 to ubiquitinate Diap1 and target it for degradation.

We were also updated on the Apaf-1-like molecules Nod1 and Nod2 by Gabriel Nunez (University of Michigan, Ann Arbor, MI, USA). Whereas Nod1 is widely expressed in various cell types, Nod2 is expressed primarily in monocytes and dendritic cells. Both Nod1 and Nod2 are activated in response to LPS and it was shown that mouse Nod2 activates NF-κB. Loss of function mutations of Nod2 are commonly associated with Crohn's disease (inflammatory bowel disease) and random mutagenesis of the LRR domain suggests that Nods may function in the cytosol of cells as sensors/receptors for pathogen components.

The inflammasome was introduced by Jurg Tschopp (University of Lausanne, Epalinges, Switzerland) as a model for caspase-1 and caspase-5 activation. Composed of NALP1, Pycard, caspase-1 and caspase-5, formation of the inflammasome results in the activation of caspases-1 and -5 and the consequential activation of IL-1β. The key to understanding what facilitates inflammasome formation lies in the structure of its constituents; they contain PYD and CARD domains. How NALPs themselves are activated remains elusive.

Guy S Salvesen (The Burnham Institute, La Jolla, CA USA) captivated the audience with an energy filled talk towards the end of the third evening, full of kinetic modeling and 3D structural analysis of caspases. The caspase-7 zymogen was revealed to be a dimer with two active sites that can be activated by cleavage anywhere within the interdomain loop and hence follows the 'booby trap' hypothesis. In contrast, upstream caspases, such as caspase-9, become activated via the 'induced proximity' model. Along the lines of this model, it was reported that, under normal conditions, caspase-9 is found as a monomer in cell extracts, but it is only the dimeric form that is active. Interestingly, via crystallographic analysis, it was found that the caspase-9 dimer contains only one functional active site. Salvesen suggested that enforced dimerization of caspase-9 is sufficient for its activity, leaving the possibility that there may be a non-Apaf-1 way to activate caspase-9.

Although several new facets of the apoptotic program were revealed under the Hawaiian sun, many still remain to be uncovered. Just as each scuba or snorkeling trip reveals the diversity and overall wonders of nature, so each of these talks led us into a new realm of possibilities in terms of cancer research. When we first arrived on the Big Island, it seemed that paradise was unchanging, but as we departed, our thoughts of death still lingered upon its shores.

Web sites of interest described at the meeting:

http://www.apoptosis-db.org/
A bioinformatics resource for apoptosis researchers.
http://www.icnet.uk/labs/downward/
An analysis of the transcriptional program induced by Raf in epithelial cells.

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