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Meeting Report

The European Death Flying Circus

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Villa Vigoni Conference on Cell Death, Villa Vigoni, Loveno di Menaggio, Como, Italy, June 11 – 13, 2001

Villa Vigoni is a complex of five villas on Lake Como built over a century ago. The main villa was reopened on the occasion of this meeting, following extensive renovations to the building as well as to its artistic content. The Italian – German Culture centre Villa Vigoni hosted the yearly reunion of European research groups working on apoptosis. In keeping with the previous workshops, the gathering was quite informal and full of discussions fostering numerous scientific collaborations among the participants. This time the emphasis was on cell cycle, DNA damage and development.

Death Pathways. A hot topic of the workshop was the session on Death Receptors and Immunity, with a specific focus on tumour immunity. For several decades immunologists have been trying to detect specific antigenic alterations that would distinguish tumour cells from normal cells, with the ultimate goal of creating therapies tailored to the biochemical properties of individual tumours. The discussion opened with the recent developments on death receptors, and was followed by the latest data on its implications for the immune system and its relationship to cancer.

One of the key foci of the meeting was the role played by the CD95 death receptor pathway during normal and disease processes.[1] Martina Müller-Schilling (Heidelberg) showed that chemotherapy-induced apoptosis involves both death receptor and mitochondrial pathways. She showed that p53 plays an essential role in upregulating CD95 death receptor expression in response to DNA-damaging drugs.[2] Expression of the death-inducing CD95 ligand, in contrast, is p53 independent and primarily regulated by transcription factor AP-1. Moreover, Müller-Schilling demonstrated that expression of CD95 ligand is redox-dependent because its promoter contains two redox-sensitive NF-κB binding sites.[3]

Thomas Brunner (Bern) presented his recent studies on the regulation of CD95L expression and apoptosis both *in vivo* and *ex vivo* in intraepithelial lymphocytes of intestinal cryptae.

In view of a potential clinical use of TRAIL for the treatment of cancer, Henning Walczak (Heidelberg) showed that TRAIL kills 50% of human tumour cell lines, but does not affect the majority of normal tissues.[4] He elucidated the molecular events of TRAIL-induced apoptosis, which, like CD95, involves the formation of a death-induced signalling complex (DISC) and the activation of caspases 8 and 3.[5] Recent results also suggest that the initiator caspase 10 can be recruited to the TRAIL receptor DISC. Interestingly, TRAIL induced apoptosis in chemoresistant tumours with high Bcl-2 and Bcl-x_L levels, suggesting that these Bcl-2 family members have less impact on TRAIL- than on chemotherapy-induced apoptosis. An extensive discussion followed about a potential hepatotoxicity of TRAIL that may limit its clinical use.

Giovanna De Chiara (Rome) described the role of MAP kinases in the signalling of memory B cells. NGF modulates B cell survival through the inactivation of p38 MAP kinase, thus preventing the proapoptotic phosphorylation of Bcl-2 and resulting in the fine regulation of cell survival.

Klaus Schulze-Osthoff (Münster) showed how keratin-18 is cleaved by caspases 3 and 6, generating a neoepitope that can be detected by a specific antibody. This antibody could become a useful tool for the detection of apoptotic cells in clinical samples. Furthermore, he showed that cytochrome *c* is not only translocated into the cytoplasm but can be released into culture fluids of apoptotic cells. Thus, cytochrome *c* release may serve as a marker for the measurement of apoptosis *in vivo*, for instance in sera of leukemic patients.[6]

In the same session some molecular pathways involved in cell death were discussed. Tissue transglutaminase (tTG or type 2) is a transamidating enzyme catalyzing the irreversible crosslinking of proteins by isodipeptide cross-linkages. The enzyme is regulated both at the transcriptional (retinoids) and post-transcriptional (GTP, Ca^{2+}, nitric oxide) level. tTG is induced and activated in cells undergoing apoptosis. Vincenzo De Laurenzi (Rome) presented the recent knockout of transglutaminase 2, with no obvious phenotype.[7] Tom Cotter (Cork) described a model of cell death in mouse retina, where light-induced cell death is mediated by nitric oxide. In this system he also demonstrated that the cell death seen occurred in a caspase-independent manner.[8]

Cell cycle and DNA damage. This session introduced the interplay between DNA damage, cell cycle arrest and apoptosis.[9] To this end, Domenico Delia (Milan) illustrated the role of APC (adenomatous polyposis coli) in colorectal

cancer and the involvement of the mismatch repair genes MSH, MLH, PMS-1 and PMS-2. ATM which is mutated in Ataxia telangiectasia belongs to the PI(3)K-related kinases. Upon double-stranded DNA breaks, the enzyme triggers cell cycle arrest by phosphorylating p53 and the checkpoint kinase Chk2. Chk2 is also controlled by Nbs1, the gene product involved in Nijmegen breakage syndrome. Delia described the role of large nuclear macromolecular complexes in controlling DNA damage responses and the cell cycle, as well as mutations of the key players in cancer development.

On the boundary between cell cycle and apoptosis, Emanuela Grassilli (Milan) discussed her data on effector mechanisms and the requirement of c-Myc in anticancer drug-induced apoptosis. Kristian Helin (Milan) showed recent data on the E2F/Rb pathway in human cancer, where Rb is mutated in about 25% of all cases. Helin used an E2F1-ER W138 fibroblast line induced by tamoxifen. E2F triggers apoptosis via a new pathway acting on Apaf-1.[10] Both p53 and E2F1 directly upregulate the promoter of Apaf-1. In fact, E2F1-induced apoptosis is impaired in Apaf-$1^{-/-}$ MEFs. In keeping with this result, the expression of Apaf-1 is dysregulated in $Rb^{-/-}$ embryos. Other genes induced by E2F are Bcl-3, caspases 3 and 7, ASK1 and cyclin E1. The induction of several apoptosis genes by E2F1 may explain why, in particular, proliferating cells are sensitive to apoptosis. This E2F-Apaf-1 pathway is parallel to another E2F1 pathway which elicits apoptosis via the direct transactivation of the p73 promoter. Gerry Melino (Rome) expanded the discussion of p53 to its family members p73 and p63.[11–13] He showed that p73 is capable of inducing apoptosis upon DNA damage. It requires the expression of the mismatch gene MLH1, and a physical interaction with c-Abl. The biochemical mechanism of this interaction is still unclear and may require phosphorylation of Tyr-99 or protein stabilisation. The expression of different p73 isoforms in normal and cancer samples was presented by Tobias Grob (Bern). In addition, he showed the cloning of a second TP73 promoter encoding a truncated protein that lacks the N-terminal transactivation domain (ΔNp73), and thus expands the complexity of this gene family. Interestingly, this promoter is driven by a p53/p73 responsive element and the ΔNp73 protein inhibits p53/p73-induced apoptosis. Therefore, ΔNp73 constitutes a dominant-negative feedback loop for both p53 and p73.[14]

Development. Michael Hengartner (Zurich) presented surprising data on the role of phagocytosis in cell killing of *C. elegans*.[15] While previously phagocytosis was considered as a final disposal event of apoptotic cells, Hengartner showed that, at least under weak proapoptotic conditions, it now appears to be directly involved in the execution process of killing. He showed that blocking engulfment genes can enhance cell survival, indicating that genes involved in phagocytosis can also function to actively kill cells. It was intriguing to see that these early phases of cell death seem to be reversible.

Francesco Cecconi (Göttingen/Rome) demonstrated through a gene trap approach that Apaf-1 is required for apoptosis induction in some cell types. He presented recent data on the involvement of apoptosis in the development of the eye and the inner ear. Martin Wagner (Ulm) described a new tumour progression model for ductal pancreatic cancer in mice overexpressing TGF-alpha under the elastase promoter.[16] Crossbreeding with p53-deficient mice dramatically accelerated tumour development in TGF-alpha transgenic mice. Deletions of the tumour suppressor loci Ink4a/Arf or Smad4 were often found in the developing tumours suggesting that these genetic events are critical for pancreatic tumour formation. Gabriella Topley (Turin) presented her recent results on mice deficient in citron kinase, a Rho-interacting kinase involved in cytokinesis. The mice show a cerebellar phenotype including severe microencephaly and epileptic seizures. Last not least, Reiner Jänicke (Münster) discussed mechanisms of bacteria-induced apoptosis in immune cells. He showed that *S. aureus*-induced apoptosis is mediated by alpha-toxin, a soluble pore-forming protein that, in addition to effects on the target cell membrane, may directly induce cytochrome *c* release.[17] This mechanism may help the bacterium to immunosuppress its host and to continue proliferating.

The meeting was financially supported by EiRx Therapeutics, the recently established Biotech Company in Cork, Ireland, dedicated to the identification of novel genes involved in apoptosis.

Altogether, this workshop, and in particular its informal part, not discussed here, fostered new cooperations between leading European scientists working on apoptosis. Indeed, this has already created important interactions[1,9,18,19] and will stimulate new collaborations. No doubt there should be follow-up meetings in the tantalising scenery of death and life.

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