Plenary session 4

Regulation of cell survival and death

PL4-1

THE ROAD MAP TO PROGRAMMED CELL DEATH: SWITCHING BETWEEN APOPTOSIS AND AUTOPHAGIC CELL DEATH

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The complexity of the molecular network underlying programmed cell death was analyzed by RNAi-mediated silencing perturbations. It was found that a few independent roads can lead to the final execution of cell death. In addition to the well known caspase-dependent pathways, the autophagic machinery can be recruited as another mechanism which causes the ultimate cell death. DAP-kinase, a calcium regulated microfilament associated Ser/Thr kinase, was identified as a key protein which can either drive a switch between apoptotic and autophagic cell death modes, or may function as a common integrator of both directions. In a search for potential substrates to this kinase we discovered a novel isoform of p19ARF, named smARF, which induces autophagic cell death. smARF is generated by a mechanism of internal initiation of translation resulting is a short isoform which lacks the nucleolar targeting signals and the p53 activating domain. smARF is a short lived protein, which is rapidly degraded by the proteasome, but accumulates after inappropriate proliferative signals generated by oncogenes.
Surprisingly, smARF translocates to the mitochondria, impairs the structure of the mitochondria, and dissipates the mitochondrial membrane potential in a p53 and Bcl-2 family independent manner. Ultimately, smARF induces massive autophagy in cells, as identified by various molecular markers and by electron microscopy. The knock down of genes which are part of the basic autophagic machinery protected to some extent the cells from death, proving that smARF induces caspase-independent autophagic cell death. Thus, in principle, two pathways can be activated. The full length nucleolar isoform of p19ARF can activate a pathway from within the nucleolus which involves p53 activation, followed by caspase-dependent type I apoptotic cell death. smARF, on the other hand, can induce a second pathway that initiates within the mitochondria, involves the dissipation of mitochondrial membrane potential, and ultimately will induce type II autophagic cell death.

PL4-2

A FUNDAMENTAL BIMODAL ROLE FOR NEUROPEPTIDE Y1 RECEPTOR IN THE IMMUNE SYSTEM AND A MODULATOR OF CYTOKINE PRODUCTION.

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Psychological conditions including stress compromise immune defences. While this concept is not novel, the molecular mechanism behind it remains unclear. Neuropeptide Y (NPY) in the central nervous system is a major regulator of numerous physiological functions, including stress.
Postganglionic sympathetic nerves innervating lymphoid organs release NPY, which together with other peptides activate five Y receptors (Y1, Y2, Y4, Y5 and y_6). Using Y1-deficient ($Y1^{-/-}$) mice, we showed that $Y1^{-/-}$ T cells are hyper-responsive to activation, and trigger severe colitis after transfer into lymphopenic mice. Thus signaling through Y1 receptor on T cells inhibits T cell activation and controls the magnitude of T cell responses. Paradoxically, $Y1^{-/-}$ mice were resistant to Th1-mediated inflammatory responses and showed reduced levels of the Th1-promoting cytokine interleukin-12 (IL-12) and reduced IFNγ production. This defect was due to functionally impaired antigen-presenting cells (APC) and consequently, $Y1^{-/-}$ mice had reduced numbers of effector T cells.
These results demonstrate a fundamental bimodal role for the Y1 receptor in the immune system, serving as a strong negative regulator on T cells potentially playing a role in T cell tolerance, but also as a key activator of APC function. Our findings uncover a sophisticated molecular mechanism regulating immune cell functions that can lead to stress-induced immunosuppression.

PL4-3

BH3-ONLY PROTEINS ARE ESSENTIAL INITIATORS OF PROGRAMMED CELL DEATH AND STRESS-INDUCED APOPTOSIS IN NORMAL AND CANCER CELLS

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Genetic and biochemical experiments have demonstrated that BH3-only proteins are essential for initiation of programmed cell death and stress-induced apoptosis and that Bax/Bak-like proteins are required for this process, probably functioning downstream. Different BH3-only proteins are required for cell death induced by different stimuli and they can also function in a cell type-specific manner. Bim is required for the death of many cell types triggered by growth factor withdrawal, for deletion of autoreactive lymphocytes and for termination of cytotoxic T cell (CTL) immune responses. The apoptosis provoked by DNA damage requires the p53 tumor suppressor and this death is dependent on the BH3-only protein Puma and to a lesser extent also Noxa.

Surprisingly, Puma was found to also be essential for apoptosis induced by several p53-independent stimuli, including cytokine withdrawal or treatment with glucocorticoids or phorbol ester. Experiments with non-transformed cells and tumour cells have demonstrated that BH3-only proteins are essential for anti-cancer therapy-induced cell killing. Puma is required for apoptosis induced by γ-radiation or several widely used chemotherapeutic drugs, including etoposide or dexamethasone. Bim, on the other hand is needed for the death of chronic myelogenous leukemia (CML) cells triggered by the BCR-ABL kinase inhibitor Glivec.