Cell Death Research at IMM and KI

Apoptosis was first introduced as a form of cell death by Kerr, Wyllie and Currie in 1972. However, this topic attracted little research attention for the following several years. In 1986 Sten Orrenius was invited to give a talk in an American-European debate on Mechanisms of Cell Injury, which was held at a Society of Toxicology meeting in New Orleans. Another speaker was Andrew Wyllie, who described their early studies of apoptotic cell death. Back in Stockholm, Sten sent a young PhD student, David McConkey, to visit Wyllie's laboratory in Edinburg to learn techniques to measure apoptosis. Upon his return to KI, David was able to show a requirement of Ca^{2+} for glucocorticoid-induced DNA fragmentation and cell death in thymocytes. Subsequently, Ca^{2+} was found to play a role in the apoptotic process in other cell types, such as lymphocytes, leukocytes, aortic smooth muscle cells, pancreatic β-cells and cerebellar granule cells, as well as in a variety of cancer and non-cancer cell lines.

In the early 1990'ies, caspases, a newly discovered group of cysteine proteases, were found to be involved in the initiation and execution of apoptosis, and KI researchers contributed to the early development of this field. Thus, we were among the first to describe the involvement of caspase-3 (CPP32/Apopain) in CD95-mediated apoptosis. In particular, Boris Zhivotovsky, who had joined the group in 1991 with a long standing experience in radiation-induced cell death from his previous work with Prof. Kaido Hanson in Leningrad, has focused on the detailed analysis of caspase-2, the most intriguing enzyme within the family, and found the importance of this non-processed enzyme for the engagement of the mitochondrial apoptotic pathway. Moreover, for the first time a functional link between caspase-2 and p53 was described.

With time became clear that all caspases in addition to be involved in apoptosis signaling fulfill normal, non-apoptotic functions. Consequently, we found two new proteins that interact with caspase-2 and described their non-apoptotic physiological functions. Important seminal paper by Bertrand Joseph's group showed that stimulation of microglia with various inflammogens activates caspase-8 and caspase-3/-7 in microglia without triggering cell death in vitro and in vivo. Bertrand Joseph made also an important discovery concerning the transcriptional and epigenetic control of autophagy.

In 1996 evidence indicating a role for cytochrome *c* in the mitochondrial pathway leading to apoptosis was presented. Importantly, long before the "apoptosis" era, radiobiologists had shown that cells isolated from radiosensitive tissues after radiation were deficient in respiration, and that this

phenomenon was associated with lower amounts of cytochrome *c*. Subsequently, in collaboration with colleagues at the University of Bergen in Norway, our group characterized in detail the apoptotic process induced by microinjection of cytochrome *c* into a variety of cell types and demonstrated that presence of the intact hemoprotein in the cytosol was enough to trigger caspase activation and apoptotic cell death. Since then, much of our work has focused on potential mechanisms of cytochrome *c* release preceding caspase activation in intact cells. Together with Vladimir Gogvadze, an expert on mitochondria, we have characterized the mechanisms of cytochrome *c* release in detail and shown the protective effect of inhibitors of either mitochondrial Ca^{2+} uptake into the mitochondria, or of pore formation in the mitochondrial membrane.

It is known that cytochrome *c* is normally attached to the outer surface of the inner mitochondria membrane by an association with the anionic phospholipid, cardiolipin. In order to better understand the mechanisms by which cytochrome *c* cleaves the mitochondria, Martin Ott, a Master Degree student in our group showed that the release of cytochrome *c* during apoptosis signaling might be a two-step process, involving detachment of the hemoprotein from oxidized cardiolipin followed by its release into the cytoplasm through Bax/Bak-induced pores in the outer mitochondrial membrane (OMM). This two-step concept of cytochrome *c* release has been supported by a host of studies.

It has long been known that mitochondria can also trigger caspase-independent apoptosis, which is dependent on the release of Apoptosis Inducing Factor (AIF) from mitochondria and its translocation into the nucleus, although the precise mechanism of this release was unknown. However, our PhD student Erik Norberg found that the release of AIF was preceded by calpain 1 cleavage of inner mitochondrial membrane-bound AIF triggered by a prolonged increase of cytosolic/intramitochondrial Ca^{2+}. Moreover, this increase was the result of the import of extracellular Ca^{2+} via hyperpolarization-activated cyclic nucleotide-gated channel 2 (HCN2). In addition to activating calpain, the imported Ca^{2+} stimulated mitochondrial ROS production leading to oxidative modification of AIF making it more susceptible to calpain cleavage. Erik was awarded with Dimitris N. Chorafas Prize for the best PhD Thesis at Karolinska Institutet.

In the seminal publication by Wyllie and colleagues in 1972, only two cell death modalities were described, apoptosis and necrosis. Recently, the Nomenclature Committee on Cell Death has characterized more than ten different types of cell death. Of interest, already in the late 1980'ies our Department of Toxicology reported that a variety of chemicals can exert their

toxicity via induction of apoptosis. Glucocorticoid-induced apoptosis was detected in thymocytes and lymphocytes, whereas 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) was able to trigger apoptosis in thymocytes. Heavy metals (Cu, Cd, MeHg, Pb), organotin compounds and dithiocarbamates were also shown to be able to kill cells via apoptosis. More recently, however, several chemical toxicants have been demonstrated to also be able to induce autophagic cell death. These include cytostatics, ionophores and oxidants, whereas ER stress is known to be able to cause apoptosis as well as autophagic cell death. TCDD and cadmium are among the environmental contaminants which can induce cell death by either apoptosis or autophagy.

Currently, it is well-accepted that cells can undergo multiple death programs, and that there are molecular switches within the programs. In 1995, Pierluigi Nicotera first demonstrated this in our Department by showing the interplay between apoptosis and necrosis in glutamate-induced neuronal cell death. He found that glutamate can induce either early necrosis or delayed apoptosis in cultures of cerebellar granule cells. Hence, after exposure to glutamate, a subpopulation of neurons died by necrosis. In these cells, the mitochondrial membrane potential collapsed, the nuclei swelled, and intracellular debris was released into the incubation medium. Neurons surviving the early necrotic phase recovered their mitochondrial membrane potential and normal energy levels, but later underwent apoptosis. These observations suggest that mitochondrial function is a critical factor that determines the mode of neuronal death in excitotoxicity. Conversely, post-apoptotic necrosis due to intracellular Ca^{2+} overload has been demonstrated in cells whose vital Ca^{2+}-extruding proteins in the plasma membrane had been inactivated by caspase cleavage during the apoptotic phase.

Although cell death research was first concentrated at Department of Toxicology/IMM, several groups from other departments have since started to also work in this field. Thus, Klas Wiman (Department of Oncology-Pathology) independently, and together with Galina Selivanova (Department of Microbiology and Tumor Biology), managed to reactivate mutant p53 and kill tumor cells by apoptosis. Sonia Lain from the same Department is focusing on the attempts to chemically increase p53 synthesis and enhance tumor cell killing by p53 degradation blockage. Thomas Perlmann (Department of Cell and Molecular Biology) has published in Nature Neuroscience an excellent story describing dopaminergic control of autophagic-lysosomal function that implicates Lmx1b in Parkinson's disease. Andrei Chagin (Department of Physiology and Pharmacology) successfully investigates importance of autophagy mechanism in bone development. Elias Arner (Department of Medical Biochemistry and Biophysics) is involved in understanding the

mechanisms of ferroptosis. Helin Norberg (Department of Physiology and Pharmacology) has published several papers on chaperone-mediated autophagy, the less known type of autophagy. Gunnar Nilsson (Department of Molecular Medicine) has investigated the mechanisms involved in regulating mast cell longevity and survival in health and disease, and identified Bcl-2-family members as crucial regulators of mast cell survival and apoptosis. Marie Arsenian Henriksson (Department of Microbiology and Tumor Biology) investigates cell death in neuroblastoma cells that involves the activity of MYCN. Nico Dantuma (Department of Cell and Molecular Biology) investigates the role of ubiquitin system in protein toxic stress-induced cell death. Dan Grander and Aris Panaretakis (Department of Oncology-Pathology) were focused on the analysis of cell death induced by chemotherapeutic drugs in acute lymphoblastic leukemia. Thus, the activity in this fast-developing area of research is significantly increased at Karolinska Institute during last several years.

It is important to note that in 1995 Boris Zhivotovsky and Sten Orrenius developed the first European educational program in cell death, which was accepted by several countries, such as Belgium, Austria, Italy, and Check Republic. The course “Apoptosis: Theory and methods” is still successfully running at KI. In 2004 the second course “Cell Death and Cancer” was introduced and is also actively pursued today. Since the beginning of the 21 century three Nobel Conferences “Cell cycle and Cell Death in Disease” were organized at KI, as well as two European Conferences on Cell Death. Sten Orrenius was elected as a first President of the European Cell Death Organization (ECDO). Boris Zhivotovsky was President of this Society in 2010-2014. Both of them received an Award from ECDO for their achievement in cell death research. Sten Orrenius, Boris Zhivotovsky and Bertrand Joseph are members of the Editorial Board of the main journal in the field: Cell Death and Differentiation.

Boris Zhivotovsky & Sten Orrenius