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History of the events leading to the formulation of the apoptosis concept

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Abstract

Histological studies of ischaemic liver injury performed between 1962 and 1964 distinguished two types of cell death: classical necrosis, and a process involving conversion of scattered cells into small round masses of cytoplasm that often contained specks of condensed nuclear chromatin. Enzyme histochemistry demonstrated rupture of lysosomes in the former, but preservation of lysosomes in the latter. Similar small round masses were also observed sparsely in normal liver. Electron microscopy showed that the small round bodies resulted from cellular condensation and budding, that they were bounded by membranes and contained intact organelles, and that they were phagocytosed and digested by resident tissue cells, including epithelial cells. In work done in association with Jeffrey Searle, the process was found to occur spontaneously in a variety of malignant tumours and to be enhanced in squamous cell carcinomas of skin responding to radiotherapy. During 1971–1972, I collaborated with Andrew Wyllie and Alastair Currie while on sabbatical leave in Scotland. The newly defined type of cell death was shown to be regulated by hormones in the adrenal cortex and in breast carcinomas. Further, review of published electron micrographs of the cell death known to play an essential role in normal development revealed the same morphological pattern. We proposed that this distinctive phenomenon subserves a general homoeostatic function and suggested it be called apoptosis.

Keywords: Apoptosis; History; Pathology

1. Introduction

In this paper I will give a personal account of the evolution of the ideas that culminated in the proposal of the apoptosis concept (Kerr et al., 1972).

2. Definition of a non-degenerative type of cell death

The story began in 1962 with my Ph.D. studies in London. My supervisor, Sir Roy Cameron, suggested I examine the cellular processes involved

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in the rapid shrinkage of liver tissue that followed interruption of its portal venous blood supply. I therefore ligated the portal vein branches supplying the left and median lobes of the liver in rats (Kerr, 1965). Within hours of the operation, patches of classical necrosis developed in these lobes around the terminal hepatic venules; that is, downstream from the point of entry of the blood supply into the liver acini. Typically, the degenerate cells in these patches were disposed of by mononuclear phagocytes. The periportal parenchyma remained essentially viable, being sustained by the hepatic artery. As the ischaemic lobes shrank, however, scattered hepatocytes in the surviving parenchyma were converted into small round masses of cytoplasm that often contained specks of condensed nuclear chromatin. These masses were taken up and digested by other hepatocytes as well as by specialized mononuclear phagocytes. Their formation was clearly indicative of cell death, but the process involved differed from necrosis in its histological appearance, in affecting only isolated cells and in not being accompanied by inflammation. Importantly, very small numbers of cells were observed undergoing the process in the livers of healthy rats.

At the time I was undertaking these experiments it was widely believed that release of lytic enzymes from damaged lysosomes was involved in the pathogenesis of cell death following injury, most investigators then equating cell death with cell degeneration. I therefore applied histochemical methods for lysosomal enzymes to the rat livers (Kerr, 1965). The results indicated that the necrosis occurring around the hepatic venules was indeed associated with leakage of lysosomal enzymes into the cytoplasm of the affected cells, though this was not an early event and was unlikely to be initiating the necrosis. Lysosomes in the small round masses of cytoplasm, however, stained discretely, and histochemistry suggested that mitochondria and ribosomes in these masses were also still intact. The second mode of cell death thus appeared to be non-degenerative in nature. The name shrinkage necrosis was suggested for it.

In 1965 I returned to my home city of Brisbane and joined the University of Queensland Pathology Department. Histological studies of liver injury produced by the toxin heliotrine showed that both types of cell death were present and lysosomal enzyme histochemistry revealed the same staining patterns as had been observed in ischaemic injury (Kerr, 1967).

3. Description of the sequence of ultrastructural changes in shrinkage necrosis

Between 1967 and 1970 I carried out an electron microscopic study of shrinkage necrosis occurring in the liver (Kerr, 1969, 1971). The earliest recognisable stage of the process was found to involve condensation of the cytoplasm and nuclear chromatin and aggregation of the compacted chromatin beneath the nuclear envelope. The small round bodies were shown to comprise membrane-bounded masses of condensed cytoplasm in which the organelles appeared well preserved. Many of the bodies also contained fragments of the nucleus, in which chromatin compaction persisted. Whilst some of the nuclear fragments were surrounded by an envelope, others were not. The lack of an envelope in some cases was later shown to be an artefact of the tissue preparative techniques used at the time. Phagocytosis and digestion of the bodies by hepatocytes as well as by specialized mononuclear phagocytes was confirmed. Extracellular bodies sometimes occurred in clusters, clearly suggesting that their formation involved budding from the surface of condensing cells. Condensed cells with surface protuberances were, however, only rarely observed. It was correctly concluded that the budding process is effected very quickly. Lastly, it was suggested that the cellular condensation and budding are likely to reflect inherent activity of the affected cells (Kerr, 1971).

4. Detection of shrinkage necrosis in malignant tumours

Early in 1970, Jeffrey Searle, who was then training as a pathologist in Brisbane, mentioned to me that he had seen extensive shrinkage necrosis in histological sections of untreated basal cell carci-

nomas of human skin. I was reminded of this when the speaker at a seminar I attended was discussing the slow growth of these tumours despite the presence of numerous mitotic figures. We decided to examine basal cell carcinomas with the electron microscope (Kerr and Searle, 1972a,b). Ultrastructurally typical shrinkage necrosis was found to be prominent, the cell fragments being ingested by carcinoma cells as well as by mononuclear phagocytes present within the tumours. When we consulted the literature, we discovered that several groups had recently concluded from kinetic studies that substantial deletion of cells must occur spontaneously in many malignant tumours. By this time we had observed shrinkage necrosis in other tumour types and we proposed that it might account for much of the deletion. That shrinkage necrosis in tumours was often most extensive near patches of necrosis obviously suggested an ischaemic cause, but it was also seen in thin tumour trabeculae, where significant ischaemia was unlikely. We quoted a seminal statement by Laird (1969) that death of both normal and neoplastic cells may be a pre-ordained, genetically-determined phenomenon. Lastly, we reported preliminary evidence that shrinkage necrosis is enhanced in squamous cell carcinomas of human skin responding to radiotherapy.

5. Formulation of the apoptosis concept

In 1970, Professor Currie, then Head of the University Pathology Department in Aberdeen, Scotland, came to Brisbane as a visiting professor. I showed him my electron micrographs of shrinkage necrosis. He said that he and Andrew Wyllie had recently seen what seemed by light microscopy to be the same process in the adrenal cortices of rats treated with prednisolone. Such treatment, of course, suppresses adrenocorticotrophic hormone (ACTH) secretion by the pituitary. The observation thus suggested that shrinkage necrosis might be regulated by hormones in endocrine-dependent tissues. I was due for sabbatical leave the following year. Currie suggested I spend it in Aberdeen. When I arrived there I was excited to learn that cell death with the histological features of shrinkage necrosis had, in fact, been detected in several sets of experiments. I embarked on electron microscopy that confirmed its identity in each case.

In addition to observing enhanced shrinkage necrosis in the adrenals of prednisolone-treated rats, Wyllie had shown that it occurs in normal neonatal rats, where there is a physiological fall in ACTH secretion. In both cases, the cell death was prevented by injection of ACTH (Wyllie et al., 1973a,b). Currie had previously studied the regression of experimental rat breast carcinomas that often follows removal of the ovaries. Re-examination of tissue from these experiments showed that the regression was associated with extensive deletion of cells by shrinkage necrosis (Kerr et al., 1972). Thirdly, Allison Crawford had shown that massive shrinkage necrosis occurring in the developing vertebral arches of fetal rats accounts for the spina bifida produced by injection of the teratogen 7-hydroxymethyl-12-methylbenz(a)anthracene into their mothers on day 11–14 of pregnancy (Crawford et al., 1972). Of greater significance for the evolution of the apoptosis concept, however, was her telling us about the extensive cell death that occurs during normal development. Knowledge of this phenomenon was largely confined, in those days, to developmental biologists. Our review of published electron micrographs of developmental death revealed the morphological features of shrinkage necrosis.

A serendipitous confluence of ideas thus made the formulation of the apoptosis concept virtually inevitable. Here we had a distinctive form of cell death with ultrastructural features suggesting an active, inherently programmed phenomenon. It played an essential role in normal development, where it was clearly precisely controlled; it was involved in cellular turnover in normal adult animals, and in endocrine-dependent tissues was under hormonal control; it accounted for both normal involution and pathological atrophy of tissues; it occurred spontaneously in malignant tumours and was implicated in tumour regression produced by at least some types of treatment. We concluded that it subserves a general homoeostatic function in regulating the size of cell populations under both normal and pathological conditions. That it could be triggered by agents that also cause

necrosis, however, was puzzling. At the time, we were completely unaware of the fact that certain components of the apoptosis concept had been explicitly enunciated many years previously, as discussed by Majno and Joris in their 1995 review.

Apoptosis initially aroused little interest. During the past decade, however, research on the process has grown rapidly (Melino et al., 2001). A comprehensive understanding of its regulation and execution may soon be within reach.

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