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Editor

Apoptosis: Biology and Mechanisms

Results and Problems in Cell Differentiation

Springer

S. Kumar (Ed.)

Apoptosis: Biology and Mechanisms

With 13 Figures

Springer

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ISSN 0080-1844
ISBN 3-540-64631-0 Springer-Verlag Berlin Heidelberg New York

Library of Congress Cataloging-in-Publication Data
Apoptosis : biology and mechanisms / S. Kumar (ed.).
p. cm. — (Results and problems in cell differentiation; 23)
Includes bibliographical references and index.
ISBN 3-540-64631-0 (hardcover : alk. paper)
1. Apoptosis. I. Kumar, Sharad. II. Series.
[DNLM: 1. Apoptosis. W1RE248X v.23 1998]
QH607.R4 vol. 23
[QH6714]
571.8'35 s—dc21
[571.9'36]

Cover Design: Meta Design, Berlin
Typesetting: Best-set Typesetter Ltd., Hong Kong
SPIN 10571118 39/3137 – 5 4 3 2 1 0 – Printed on acid-free paper

Results and Problems in Cell Differentiation

Series Editors:
W. Hennig, L. Nover, U. Scheer

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Preface

Apoptosis is currently one of the fastest moving fields in biology with spectacular progress made over the past few years in delineating the molecular mechanisms which underlie this process. It is now indisputable that apoptosis plays an essential role in normal cell physiology and that aberrant apoptosis can manifest itself in a variety of human disorders. Published in two parts (Volumes 23 and 24 of the series entitled *Results and Problems in Cell Differentiation*), this is an attempt to bring together many different aspects of apoptosis. Given that this is such a vast and rapidly expanding field, it is almost impossible to cover everything that is now known about apoptosis in two short books, but I hope these volumes prove to be a guidepost, providing basic essential information on the biology and molecular mechanisms of apoptosis and its implications in some human diseases.

As a significant amount of new information on apoptosis is emerging every week, it is unrealistic to expect that by the time these two books are published, all the articles will deliver up-to-date information. Nevertheless, I believe that the fundamentals of the apoptotic phenomenon are now firmly in place and are discussed at length in various chapters. Readers may find a small degree of overlap between some chapters. This was unavoidable since closely related areas of apoptosis research have been covered by more than one author.

Such an endeavour would not have been possible without the help of many distinguished scientists who contributed the articles assembled in these books. I am very greateful and indebted to all the authors who made considerable efforts to submit their manuscripts as soon as they could. I am also thankful to many other colleagues and members of my laboratory for various suggestions. I have thoroughly enjoyed reading various contributions and have learnt a great deal in the process of compiling these volumes. I hope the readers will find these books a useful resource for both teaching and research purposes.

Adelaide, May 1998 *Sharad Kumar*

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A Personal Account of Events Leading to the Definition of the Apoptosis Concept

John F. R. Kerr

1
Introduction

In this chapter I will describe the sequence of events that culminated in the proposal of the apoptosis concept (Kerr et al. 1972). Since it is now many years since these events took place, it is possible that memory has become coloured by subsequent insights. The main steps in the story were, however, recorded in publications written at the time.

2
Delineation of Two Types of Cell Death with Distinctive Lysosomal Changes

I first developed a special interest in cell death when I went to London in 1962 as a young Queensland medical graduate specializing in pathology to undertake a PhD under the supervision of Professor Sir Roy Cameron in the Department of Morbid Anatomy, University College Hospital Medical School. Cameron was born and educated in Australia and, perhaps because of this, he actively fostered academic links with British Commonwealth countries; a long procession of young pathologists from the Commonwealth obtained their research training in his Department. He suggested that I study the effects on liver tissue of interrupting its portal venous blood supply, repeating experiments conducted many years previously by Peyton Rous (Rous and Larimore 1920), the same Peyton Rous who had subsequently become famous for his discovery of virus induction of tumours. He and Larimore had shown that obstruction of portal vein branches supplying several lobes of the liver resulted in rapid and marked shrinkage of these lobes with simultaneous compensatory enlargement of the rest of the organ. I therefore ligated the portal vein branches supplying the left and median lobes of the liver in rats and studied the resulting changes microscopically (Kerr 1965).

The shrinkage of the ischaemic tissue was found to be due to two distinct processes. Firstly, within a few hours of operation, confluent necrosis devel-

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oped in circumscribed areas around the terminal hepatic venules (that is, in areas furthest from the blood supply) and these groups of necrotic cells were removed by mononuclear phagocytes, the involved areas undergoing collapse. Secondly, in the periportal parenchyma, which remained viable as a result of still receiving a blood supply from branches of the hepatic artery, scattered individual liver cells were progressively deleted by a process that was morphologically quite different from necrosis. The affected cells were converted into small round or ovoid cytoplasmic masses, which often contained one or more specks of condensed nuclear chromatin. These masses were phagocytosed by Kupffer cells or by intact parenchymal epithelial cells. The process was prominent during the period of rapid shrinkage of the ischaemic lobes, and thereafter decreased as the lobes approached a new equilibrium with their residual blood supply. Importantly, the same process was found to occur at a very low rate in the livers of healthy rats; a statement to this effect is buried rather inconspicuously on page 422 of the 1965 paper.

At the time I was starting my experiments, there was a good deal of interest in the possible involvement of lysosomes in the production of cell death following various types of injury (de Reuck and Cameron 1963). These organelles had been defined only a few years previously by de Duve and his colleagues and it had been suggested that injurious agents might cause release of their digestive enzymes, thus killing the cell, an idea encapsulated in the term "suicide bag". Professor J. F. Smith, Cameron's deputy, introduced me to recently developed histochemical methods for demonstrating the distribution of lysosomal enzymes in tissues and I applied these to the rat livers (Kerr 1965). In normal animals, strings of discretely stained lysosomes were observed in the paracanalicular cytoplasm of the parenchymal cells, these organelles being normally concentrated in paracanalicular locations. In the areas of confluent necrosis, on the other hand, the affected parenchymal cells showed diffuse paracanalicular staining, which did indeed suggest release of lysosomal enzymes. The diffuse staining was, however, not an early change and it seemed likely that lysosome rupture was merely part of the general cellular degeneration that accompanies necrosis rather than being the initiating event in the production of cell death. But the most interesting finding was that the lysosomes in the small round and ovoid cytoplasmic masses were stained discretely by several different histochemical methods, indicating that they were still intact. Moreover, histochemical staining by appropriate methods suggested that ribosomes and mitochondria were also preserved in the rounded cytoplasmic masses. It was proposed that the cellular shrinkage that leads to their formation might be effected by autophagocytosis, with cytoplasmic components being progressively digested within the cell's own membrane-bounded lysosomes. This hypothesis was later refuted by electron microscopy (Kerr 1969).

At this time it seemed clear that the small cytoplasmic masses represented a distinctive type of cell death, which differed from classical necrosis in its

histological appearance, in not being degenerative in nature, in affecting only scattered single cells and in not being accompanied by inflammation. The name shrinkage necrosis was suggested for it (Kerr 1965).

At the beginning of 1965 I returned to Brisbane and took up a position in the University of Queensland Pathology Department. My first project was to apply the histochemical methods for lysosomal enzymes that I had used in London to the livers of rats given the pyrrolizidine alkaloid heliotrine, an hepatotoxic agent that produces zonal necrosis rather like that seen in the liver lobes deprived of their portal venous blood supply (Kerr 1967). Cells undergoing shrinkage necrosis were numerous in the viable parenchyma between the areas of necrosis. The staining patterns for lysosomal enzymes in the two types of cell death were found to be the same as those seen previously in ischaemic injury (Kerr 1967).

3
Definition of the Sequence of Ultrastructural Events Involved in Shrinkage Necrosis Occurring in the Liver

In 1967, the Queensland University Pathology Department acquired its first electron microscope and I embarked on a systematic study of the ultrastructural events involved in the evolution of shrinkage necrosis in the rat liver with the help first of David Collins and later of Brian Harmon (Kerr 1969, 1970, 1971, 1973). The stereotyped morphological sequence that emerged at that time was subsequently confirmed in many other tissues. The appearances were noted to be consistent with several recently published electron microscopic studies of so-called acidophilic or Councilman-like bodies found in the liver in naturally occurring diseases (Biava and Mukhlova-Montiel 1965; Klion and Schaffner 1966; Moppert et al. 1967). These latter bodies clearly represented the same process as I had referred to as shrinkage necrosis in 1965.

In my electron microscopic studies of rat liver I found that rounded bodies that still lay free in the extracellular space comprised membrane-bounded fragments of condensed parenchymal cell cytoplasm in which the closely packed organelles were well preserved. Some of these bodies contained masses of condensed chromatin, which only occasionally appeared to be surrounded by membranes. This lack of bounding membranes was subsequently shown to be an artefact resulting from the electron microscopic preparative techniques used in those days. It is now known that nuclear fragmentation in apoptosis is associated with preservation of the nuclear envelope (Kerr et al. 1995). The earliest nuclear changes, with condensation and margination of chromatin prior to fragmentation, were described in these early liver studies, but were seen only infrequently. The fact that the condensed cytoplasmic masses often occurred in clusters, taken in conjunction with the extremely small size of some of the masses, indicated that they arose by a process of budding-off of

protuberances that developed on the surface of condensing cells. The actual process of budding was, however, rarely observed. This was correctly interpreted as indicating that it occurs very quickly (Kerr 1969), a conclusion that appeared to be supported by the time-lapse microcinematographic observations of cell death taking place in vitro that had been made by Marcel Bessis (1964). Bessis's classical contributions to the understanding of cell death have recently been reviewed by Majno and Joris (1995). Phagocytosis of the condensed, membrane-bounded liver cell fragments by Kupffer and parenchymal epithelial cells was confirmed and their progressive degradation within phagolysosomes was followed by electron microscopy.

In a paper submitted in November 1970 (Kerr 1971) it was suggested that, whilst severe damage to a tissue causes classical necrosis, a moderately noxious environment induces scattered cells to undergo shrinkage necrosis. Secondly it was stated that shrinkage necrosis constitutes one type of cell death occurring in normal tissues. Thirdly it was concluded that the prolific cellular budding that occurs in shrinkage necrosis is likely to be the result of inherent activity of the cells themselves. However, it was noted that the involvement of only scattered, individual cells by the process would make its biochemical analysis difficult.

4
The Occurrence of Shrinkage Necrosis in Tumours

About the middle of 1970, I attended a seminar on tumours. I remember the speaker discussing the slow growth rate of basal cell carcinomas of human skin, which appeared paradoxical in view of the high rate of mitosis observed within them. I was reminded of the fact that Jeffrey Searle, who was then training as a pathologist at the Royal Brisbane Hospital, had recently pointed out to me that cells with the histological features of shrinkage necrosis were often numerous in these tumours. We decided to undertake a light and electron microscopic study of surgically excised basal cell carcinomas, which are particularly common in Queensland.

In a paper submitted in July 1971 (Kerr and Searle 1972a) we confirmed that shrinkage necrosis could be found in all basal cell carcinomas and that it was often extensive. The ultrastructural appearances were similar to those seen in the liver. Many of the condensed cell fragments were taken up and digested by tumour cells.

When we looked at the literature, we found that it had recently become apparent that there is a marked discrepancy between the rate of growth of a variety of malignant tumours and the rate of division of their constituent cells. On the basis of these findings, a number of investigators had concluded that spontaneous loss of cells is often a significant determinant of tumour growth rate. However, the mechanism of the loss was not understood.

We found that the extent of shrinkage necrosis in parts of some basal cell carcinomas was comparable to that seen in the liver lobes deprived of their portal blood supply; the latter decreased to about one-sixth of their original weight within 8 days of operation. Cellular deletion by shrinkage necrosis in basal cell carcinomas was thus likely to be very significant kinetically. Further, we found that extensive shrinkage necrosis could also be detected by light microscopy in other types of malignant tumours. We suggested that this process makes a major contribution to cell loss occurring in untreated tumours in general (Kerr and Searle 1972a). We also speculated about its possible causes. Whilst the fact that it was often prominent near the centres of large tumour nodules suggested that it was caused by mild ischaemia, we sometimes saw it in thin tumour trabeculae that were no more than two cells thick. Significant ischaemia was unlikely to be present in such trabeculae. By this time we had seen shrinkage necrosis in many of the tissues of healthy animals and had concluded that it is involved in normal cellular turnover in these tissues (Kerr and Searle 1972a). We quoted the seminal statement by Laird (1969) that death of both normal and neoplastic cells may be a pre-ordained, genetically determined phenomenon.

In a second study (Kerr and Searle 1972b) we confirmed by electron microscopic histochemistry that fragments of cells that had undergone shrinkage necrosis in basal cell carcinomas and that had been phagocytosed by viable tumour cells were degraded within lysosomes. We also reported preliminary observations indicating that the extent of shrinkage necrosis in squamous cell carcinomas of human skin increases in response to radiotherapy.

5
Proposal of the Apoptosis Concept

In late 1970, Professor A. R. (subsequently Sir Alastair) Currie, who at that time was Head of the Department of Pathology in the University of Aberdeen, Scotland, came to Brisbane for a month as a guest professor in the University of Queensland. I showed him electron micrographs of shrinkage necrosis occurring in the liver and discussed the plans to study basal cell carcinomas. He was intensely interested.

Currie had a major interest in endocrine pathology and had long mused upon the reversible increase and decrease in the size of endocrine-dependent tissues that follows changes in blood levels of trophic hormones; he had studied the regression of rat breast tumours induced by 9,10-dimethyl-1,2-benzanthracene (DMBA) that often follows surgical removal of the ovaries; and with Andrew Wyllie, who had recently commenced PhD studies under his guidance, he had observed scattered, single, dead cells by light microscopy in the adrenal cortices of animals in which adrenocorticotrophic hormone (ACTH) secretion had been interrupted (Wyllie 1994–1995). He was excited by the possibility that the ultrastructurally distinctive type of cell death I

had shown him might be regulated by trophic hormones in endocrine-dependent tissues. I was due to take study leave from the University of Queensland the following year. He suggested that I spend it in his Department in Aberdeen.

As a side issue during his treatment of rats with DMBA to induce breast tumours, Currie had noticed single cell death in the adrenal cortices of some animals; in others there was confluent adrenal cortical necrosis. At his suggestion, I studied these lesions before going to Aberdeen. The single cell death was found to display the ultrastructural features of shrinkage necrosis (Kerr 1972).

Following my arrival in Aberdeen in September 1971, electron microscopy was performed on tissues obtained from several series of animal experiments that had been under way in the Pathology Department there for some time. In each case, single cell death with the ultrastructural features of shrinkage necrosis was found to occur.

Andrew Wyllie had shown that decrease in the size of the adrenal cortex that follows experimental interruption of ACTH secretion in both adult and fetal rats is accompanied by death of many scattered epithelial cells in the inner part of the cortex, and that similar cell death also takes place in normal neonatal rats, where adrenal cortical shrinkage is associated with a physiological decrease in ACTH secretion. In all cases, he had found that the cell death was prevented by coincident injection of ACTH. Two papers describing the occurrence and the ultrastructural features of the adrenal cell death (by then referred to as apoptosis) were returned by the editors of endocrinological journals with scathing referees' comments (Wyllie 1994–1995); they were subsequently published in the *Journal of Pathology* (Wyllie et al. 1973a,b).

Histological sections and blocks of tissue that had been processed for electron microscopy were available from the experiments carried out by Currie and his colleagues on DMBA-induced rat breast tumours. These were restudied. Large numbers of tumour cells were observed to undergo shrinkage necrosis when regression followed removal of the ovaries (Kerr et al. 1972).

At the time of my visit to Aberdeen, Allison Crawford, a developmental biologist, was working in the Pathology Department on the teratogenic effects of 7-hydroxymethyl-12-methylbenz(a)anthracene, one of the principal metabolites of DMBA. Injection of this substance in Sprague–Dawley rats on day 11–14 of pregnancy resulted in the occurrence of encephalocoele and spina bifida in the mature fetuses. She had found that these developmental defects were explicable on the basis of tissue deletion resulting from massive but localized single cell death occurring within 24 h of injection of the teratogen. Electron microscopy performed on her experimental fetuses showed that the ultrastructural features of this death were those of shrinkage necrosis (Crawford et al. 1972). Of perhaps even greater importance to the evolution of the apoptosis concept, however, was the fact that she introduced us to the