Book Review

When cells die II: a comprehensive evaluation of apoptosis and programmed cell death

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When cells die II: a comprehensive evaluation of apoptosis and programmed cell death, edited by RA Lockshin and Z Zakeri. Wiley-Liss (wiley.com), New Jersey; 2004. ISBN 0-471-21947-9

Usually, when the sequel of a Hollywood blockbuster is going to be released and overpriced merchandizing is flooding the planet you think – eh, part two can never be nearly as good as the first one. And usually you are absolutely right. Just think about 'Matrix-Reloaded' or to get closer to our subject 'Die Hard II', although I really liked the scene where Bruce Willis ejects himself out of this grounded fighter-jet. However, interestingly, we cannot wait to get to the cinema and – to get disappointed – but, hey, what were we expecting? Not even Hollywood can meet all these anticipations raised in our heads after having seen part one. The only exception of the rule, according to one of my students, seems to be 'Lord of the Rings'.

The good thing about Hollywood sequels is the fact that you hadn't have to see part one in order to get the story line of part two, the exception, again, being 'Lord of the Rings', probably. Anyhow, to stress my favorite example again, you really didn't have to see 'Die Hard' in order to get the plot in 'Die Hard II'. However, in contrast to the director of this notorious Hollywood movie, it really mattered to the editors, or lets call them also directors, of 'How Cells Die I+II' why and, most importantly, how their leading acts – in their case the cells in our own body or other model organisms – die.

In editing this book Richard Lockshin and Zahra Zakeri tried to fulfill a sheer impossible task that is, providing the novice in this field of research as well as the senior investigator still working in the field with a comprehensive textbook on apoptosis that should satisfy the needs of both. So the question is: can these different expectations be met. If you are, lets say a Ph.D. student, working on a subject dealing with apoptosis you would be happy if someone tells you 'You do not have to read these 82 140 papers listed in PubMed when you type in 'apoptosis or programmed cell death' (uhps, its already 82 141 papers while I typed this sentence) – just have a good look through Richards and Zahras book here – you will find all the necessary information there'. Or, lets assume you are a young investigator just trying to set up your own lab because you have done a postdoc dealing with apoptosis, for example, Bcl-2 or related matters and you are still fascinated by the subject. You realize you can't read every interesting paper (uhps 82 142) because all of a sudden you got additional stuff to do, things you never thought of during your postdoc days, but you want a comprehensive overview on cell death in *Drosophila*, for example, or refresh your memory on IAPs (inhibitor of apoptosis proteins – for the novice). Maybe you ask yourself 'what is actually the textbook view on this

controversial issue everybody was talking about 2 or 3 years ago – you name it, e.g., the *harlequin* mouse. Maybe you are also a senior investigator and you want to find out whether the discoveries you and your lab members made over the past decade passed the test of time and made it into the textbooks or whether people forgot all about your contributions already. Life as a scientist is tough some times, or actually, most of the times.

Richard Lockshin and Zahra Zakeri made quite an effort to cover all possible fields and aspects related to apoptosis research by convincing a large number of reputed investigators with a long-standing track record in the field to contribute to the second part of 'When cells die'. The books starts off with an interesting brief history lesson by Richard Lockshin and Zahra Zakeri explaining how cell death research developed, what forms of cell death we know of to date, how these forms of cell death may occur in brief, for example, caspase-dependent or -independent death, autophagic cell death or necrosis – although it was news to me that cytochrome *c* displaces AIF in order to activate caspase-9. What a difference two letters can make. For sure the editors overlooked this typo and since we have learned that cell death in most cell types and organisms can occur in the absence of these molecules it doesn't really matter.

This also tells me that it is very hard for a book to be up to date since most fields of research are moving so fast that the models and concepts that were hot 2 years ago may have been disproved or replaced by others already – an inherent imperfection of textbooks that we have to live with as readers. Richard and Zahra continue and give a nice introduction on the role of apoptosis during embryogenesis in general, lining out its importance for the early development of the neuronal as well as the immune system. Personally, however, I do not believe that autoimmunity in Fas-deficient *lpr* mice is caused by defects in negative selection in the thymus of these animals as mentioned in the text. They also sum up a large body of information gained from various mouse gene knockout studies where deletion of certain pro- and antiapoptotic molecules, for example, caspases and Bcl-2 family members, respectively, led to detectable embryonic phenotypes underlining their role in vertebrate development – certainly a good summary interesting to the more experienced researcher in the field and they finish off with a detailed overview on various methods used to analyze and detect cell death, which is certainly of interest to the novice in the field.

The next chapter by Pierre Golstein takes us back to the very beginning of evolution and we are taught that even very primitive organisms such as the acrasiomycete, *Dictiostelium discoideum*, neither being plant nor animal nor a real fungus, utilizes a biological mechanism reminiscent of apoptosis during its interesting life cycle. Usually these creatures live as solitary haploid amoebae in the soil but tend to form aggregates if food (bacteria) is limited. Under these conditions, the individual amoebae can act as a unit in order to form a structure called the sorocarp. The sorocarp looks more or less like an old-fashioned lampshade in a 1950s living room, if you look it up in a textbook. This lampshade structure consists out of a fruiting body full of spores on top of a stalk that consists out of cells that are considered dead because they cannot be regrown. Presumably, some amoebae in this aggregate underwent a form of developmental cell death in order to produce this structure. Using this model system, maybe, one day we can really understand how programmed cell death or apoptosis evolved and what the very first players on the field were.

I was really looking forward to read the next chapter, making a quantum leap forward in evolution the reader becomes confronted with the immense complexity cell death signaling reached in an organism still considered 'simple' by vertebrate biologists, that is, the fruit fly. Loretta Dorstyn and Sharad Kumar did a really great job in explaining and summarizing what is known currently about apoptosis signaling in *Drosophila*. As far as I can tell, the chapter is also very up to date since some citations from the year 2003 can already be found. Pretty good for a book published not even 1 year later. They nicely point out the biological role and features of key players only found in *Drosophila* cell death signaling, for example, the RHG proteins reaper, hid and grim as well as sickle (jafrac2 wasn't known these days), where true mammalian homologues have still not been found. One of those initially thought to be a functional orthologue, Htra2/Omi, has been shown in genetic studies to be dispensable for cell death to occur and may even have antagonistic functions in normal physiology as exemplified in the *mnd2* mutant mouse. I also had to realize that there are already seven caspases known in *Drosophila*, as opposed to 12 in mouse and man. The last time I had a good look at that issue there may have been four *Drosophila* caspases, but please, don't blame me for that. Taken together, the chapter gives you a good overview, regardless whether you are a Ph.D. student starting to work on *Drosophila* cell death or an investigator working on cell death in a different model organism. Personally, I always envied *Drosophila* geneticists for their generally accepted freedom to give genes and proteins the most funkiest names, usually leaving the less informed reader with no clue what the molecule behind the name could be or do. Most of the times you cannot even make an intelligent guess e.g., why can't Eiger and Wengen simply be called dmTNF and dmTNF-R?

Next stop – another universe: programmed cell death in plants is the topic of the following chapter and if you recall that the word apoptosis was derived from ancient Greek, meaning more or less 'falling off' such as leaves do from the tree, you immediately realize that this process must play an important process in a plants life as well, if you didn't know it already.

Just think about the generation of xylem structures that are necessary to transport water and nutrients from the roots to the shoots or petal senescence. Plants also use programmed cell death to limit the spread of invading pathogens or in response to environmental stress, just as we do.

Returning from the plant universe you find yourself confronted with a 'rather simple' creature of the animal kingdom, but an excellent model system to study developmental cell death, that is, *Xenopus laevis*. Metamorphosis of the tadpole to the frog is associated with the restructuring of various organs, for example, the small intestine and the absorption of certain tadpole body features such as its tail. Interestingly, apoptosis during metamorphosis seems to depend largely on thyroid hormone (TH), which signals via binding to the thyroid hormone receptor (TR), a member of the nuclear receptor superfamily. The response to TH (DNA synthesis or cell death) is strongly modulated, for example, by the intestinal epithelial cell's interaction with extracellular matrix molecules that are able to prevent anoikis – a form of cell death caused by the loss of substrate attachment or integrin signaling. Hormones that signal via nuclear receptors, such as TH, initiate a very complex cellular response by inducing and/or repressing a large number of target genes and seem to play a crucial role during development, not only in frogs but throughout the animal kingdom. In *Drosophila*, on the one hand, the steroid ecdysone is critical for developmental metamorphosis of the fly embryo and glucocorticoids in the mammalian system, on the other hand, they are key regulators shaping the immune system – the topic of the next chapter.

I hoped to find an up-to-date view of cell death in the immune system, which is one of my prime interests but was somewhat disappointed. The chapter may give the interested student or nonspecialist a nice introduction to the topic, but due to the fact that most citations are at least 3 years old it simply lacks the cutting edge. For example, all the exciting findings identifying the BH3-only protein Bim as the key molecule executing cell death during negative selection of lymphocytes (acting upstream of Bax and Bak) are neither mentioned nor are the findings that the same cell death gene is required for deletion of activated peripheral lymphocytes to end an immune response and, subsequently, to preclude autoimmunity. Also the sections about p53, Rb and c-Myc are kept very general and not set into context to the topic of the chapter at all.

Imagine how smart we all could be, if we didn't loose so many of our neurons during development? Probably, we would just run around with much bigger heads. Targeted deletion of the key signaling elements of the apoptotic machinery caspase-3, caspase-9 or Apaf-1 in mice led to most dramatic phenotypes in the central nervous system characterized by an immense neuronal hyperplasia during early brain development. These studies and subsequent experiments demonstrated that these molecules act in a hierarchical order to induce apoptosis, indicating an epistatic relation in certain but maybe not all cell types (neurons *versus* hematopoietic cells). The chapter on neuronal cell death also discusses the role of p53-induced apoptosis in the pathogenesis of neural tube closure defects, the interrelation of Bcl-X_L or Bax with the above-mentioned signaling elements in

developmental cell death in the brain and highlights the role of Akt/PKB, JNK and NGF, as well as neurotrophins in programmed cell death of neurons.

The role of apoptosis in regulating tissue homeostasis, one of the first functions mentioned in any apoptosis review, is exquisitely explained in the following chapter written by JW Wilson and CS Potter. The following pages deal primarily with the molecular mechanisms regulating the complex biological interactions necessary to keep the balance between proliferating stem cells in the crypts of the small intestine and colon and continuous cell death of mature differentiated epithelial cells at the table of the villi. As you can imagine this is a very complex process and so I felt sometimes a bit lost in the large number of signaling pathways highlighted in this chapter that may directly or indirectly contribute the maintenance of tissue homeostasis in the intestine. However, I really enjoyed the chapters about the response of intestinal epithelial cells to injury and the role of apoptosis as a barrier against inflammatory and malignant diseases in this organ.

The next chapter may be of particular interest for the initially mentioned senior investigators since it deals with ageing and the relation of this unavoidable phenomenon to apoptosis.

Having said this, I hope I can still convince all of you to stay with me for the second half of the book (review) – don't give in – I know, like in all part two Hollywood movies it gets a bit lengthy in the middle, but the best is yet to come, trust me (usually a stunning chase across town with lots of explosions, a huge car pile up, an unnecessary love scene before the hero saves the day and we can leave the cinema with a strange gut feeling wondering how part three will continue and when it will be released – or probably the gut feeling arises because our stomach is full of coke, pop corn and nachos with cheese).

So let us start with Section 3 'How cell death is carried out' opening with a chapter called 'survival factors' contributed by the man who realized first that Bcl-2 is not a true oncogene promoting proliferation, but a survival molecule preventing cell death and that the same molecule can substitute for Ced-9 in the worm, among many other things. I wonder if he still has this beehive in his office where the walls are plastered with schemes of apoptosis pathways that he believes are bogus for various reasons. Keeping this in mind the following chapter is surprisingly balanced and gives a coherent overview how cell death may be carried out, focusing on the intrinsic cell death signaling cascade, giving every possible scenario a fair perspective of being the right one. Too bad it is just a very short chapter, as opposed to the next one where the reader is confronted with an unwelcome truth. There is something else than apoptosis or necrosis, for example, autophagy, paraptosis or caspase-independent death. Why are things so complicated? Or could you also ask: do some of these mechanisms really exist as physiological responses of cells to certain biological signals *in vivo*, or are some of these phenomena artificial because we scientists tend to twist the system to a degree that may be beyond any physiological situation?

So the movie continues and we reach a stage of the chase where lots of dead bodies are around and you need to call 'the cleaner' – you remember, the guy in 'Pulp Fiction' who lets the car with the dead body with the big hole in its head disappear without a trace. In the mammalian system, the cleaner is

usually a macrophage and the following chapter deals with phagocytosis. It starts off with a long, long list of abbreviations, which I think is a good idea, but you also know it is going to be a complex story – but told in an easily understandable manner. The chapter tells all the tricks of 'the cleaners' and you realize that there are many different ways to make the dead body disappear. There is the metal-press that converts the car with the dead body in the trunk into one cubic foot of reusable iron. The concrete boots and the harbor and, often used by professional cleaners such as Mr. Mac'rophage, the acid tank where the body gets completely dissolved and disappears without a trace. Leaving traces behind can mean big trouble such as autoimmunity.

In my point of view the following chapter is a little bit displaced in this book. It deals with cell cycle and carries the title Cell Cycle Genes: pRb and p53. No doubt these two proteins are key regulators of cell cycle progression, arrest and/or apoptosis induction, but the chapter mainly deals with yeast cell cycle genetics at a very high-quality level and does not really link this information to programmed cell death. The main acts introduced in the title, pRB and p53, are only mentioned in the last two or three pages or so and to an extent where any general review on these proteins gives you more information.

The next chapter gets us back on track – mitochondria and their role in PCD are on the agenda. Various concepts of how mitochondrial integrity is breached during apoptosis, how cytochrome *c* and other factors may get released from mitochondria and what role cytochrome *c* may play in PCD of cells from different model organisms are discussed. Since this is an area of apoptosis research where generally accepted concepts are still missing, it is interesting to read about the pros and cons for the various models that have been proposed so far.

I reached the final section of the book 'Deregulation of cell death in disease and future intervention' and I am curious to read about a proteomics approach to identify and characterize cell death regulators contributed by Peter Vandenabeele and co-workers. Many of us owe him big thanks for organizing this great beer-tasting event at the last European Cell Death conference in Ghent. Again, there is a helpful list of abbreviations at the beginning – good on you! If you are not a biochemist or strongly interested in protein chemistry and always wondered what these proteomic approaches can be used for and what the basic principles of techniques such as mass spectrometry or MALDI-TOF are, then this is your chapter – it tells you the basics and some good examples as to how some of these techniques were used to identify key players in cell death-inducing signaling complexes such as FADD and caspase-8 in the DISC.

Marie Hardwick and co-workers take over the script and since the chapter deals with cell death in viral infections, I wonder why there has never been an Outbreak part two in the cinemas. On the other hand, if you have seen this movie you know why. It was really badly scripted as opposed to this chapter of the book. Anyway, much of our understanding of how programmed cell death works in mammals has been derived by analyzing viral infection and the responses of the target cell against the invader as well as the identification of tools viruses evolved to avoid suicide of the host cell. Just

think about all the caspase inhibitors P35, CrmA, OP-IAP, vFLIP or the Bcl-2 homologues E1B 19K-, KS-Bcl-2- and p53-neutralizing proteins, such as SV40 large T antigen or the HPV protein E6.

Inactivation of p53 or its function is also a hallmark of tumorigenesis, as we all know, and the role of cell death in cancer and cancer therapy is discussed in the following chapter by Simone Fulda and Klaus Michael Debatin who are working on this topic since ages, or at least ever since I can remember. As you can imagine, cancer cell biology is very complex and heterogeneous and definitely different from 'normal' cell biology, so cell death and survival signaling pathway may differ somewhat in transformed cells when compared to nontransformed cells, and this fact is highlighted in this chapter among other things. On various occasions, however, I wanted to track down the relevant citations giving rise to certain statements made in the text that made me curious but, unfortunately, I could hardly ever find the relevant primary citation since, in many cases, only another review was cited.

The final cut of the book is a detailed analysis of apoptosis during myocardial infarction and heart failure. What I really liked about this final chapter is the fact that it does not start off (again) with a summary of the cell death signaling pathways we know of to date, but that it highlights the clinical relevance of the issue and gives the noninformed reader a good introduction into the pathophysiology of myocardial infarction and heart failure. Kitsis and co-workers cautiously point out the role apoptosis may play in these complex pathological

conditions and provide convincing evidence that inhibition of myocyte apoptosis may be a useful therapeutic approach to prevent or treat heart diseases.

So, we finally made it to the very end of the book and I have to think about some appropriate concluding remarks. First of all, it is not really a classical 'textbook' but a collection of good reviews put together in textbook-like manner and order. In my point of view, the book is definitely a good source of information for those who work in the field of apoptosis research and of great help to those who work in related fields and want to get a good overview of what is going on in cell death research. It may also be very helpful for the novice in the field or the Ph.D. student who needs to look up some key facts quickly. However, I definitely miss some chapters needed in order to fulfill the prediction of the title 'A comprehensive evaluation of apoptosis...', since there is no chapter on caspases and how they function nor on the TNF receptor family. Also, it could have been streamlined a little bit more – as I said you will often find redundant introductions on how cell death may occur and what the key players are. Probably the editors should urge the individual author to avoid unnecessary redundancies in their introductions considering that something like 'The molecular mechanisms of cell death' has already been presented in their very first chapter of the book. However, this is just an idea of mine. So, I am curious what will come next and I agree with my student who claims that sometimes part three is the best one, citing again Lord of the Rings 'Return of the King' as an example (if there wasn't the Hobbits sheer endless journey home).