TIMELINE

Apoptosis and cancer: the genesis of a research field

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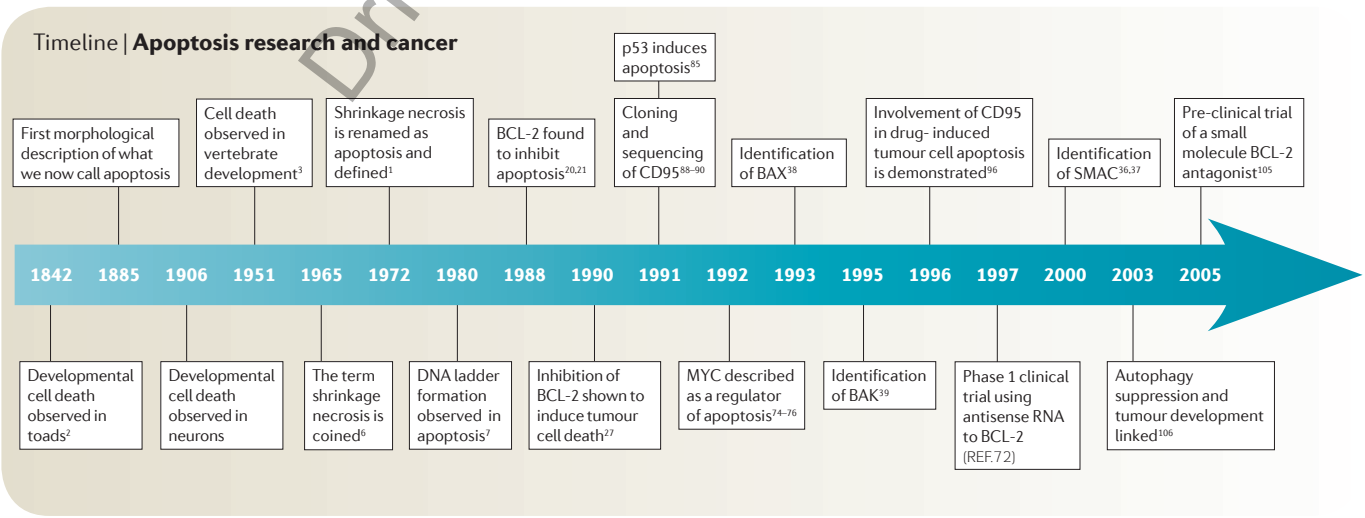
Abstract | In multicellular organisms, the total number of cells is a balance between the cell-generating effects of mitosis and cell death that is induced through apoptosis. A disruption of this delicate balance can lead to the development of cancer. This Timeline article focuses on how the field of apoptosis biology has developed in the context of its contribution to our understanding of cell death, or lack of it, in the development of malignant disease. It traces the course of research from key discoveries in fundamental biology to potential therapeutic applications.

Entering the words apoptosis and cancer into the PubMed database yielded over 62,000 references, which scared me a little as I sat down to write this Timeline article. How was I to make sense of this maze of information in the context of the development of the field? However, by pinpointing several seminal publications I began to remember how different branches of the field evolved over time, and using these publications as starting points made my job somewhat easier. It was also fascinating to delve into the history and the people behind the work. Ever since their first publication, Kerr, Wyllie and Currie have been inextricably linked to apoptosis and the development, progression and treatment of cancer. The fact that their groundbreaking paper[1] was published in a cancer journal (the *British Journal of Cancer* in 1972) set the scene for what followed on the role of apoptosis in cancer biology. The discovery of the Bcl-2 protein family, as well as p53 and its involvement in regulating apoptosis, and the CD95 (also known as FAS or APO1) pathway, led to an understanding of the molecular web that controls tumour cell death and survival. This Timeline article seeks to unravel some of this complexity and provide an insight into how and when key discoveries were made, the people behind them and the consequences of these discoveries for our understanding of the relationship between apoptosis and tumour development.

A little piece of apoptosis history

Biology is littered with examples of the importance of discoveries not being appreciated when the original observations were made. However, no major field of biology has been rediscovered as often as the field of apoptosis, which has been independently described at least five times over the past 150 years, as far as I can determine. The study of apoptosis has a history dating back to the mid 1800s (TIMELINE). The concept of natural cell death was originally mentioned in 1842 by Carl Vogt[2] after he studied developmental cell death in toads — a remarkable piece of science given that the cell theory of life was established only 3 years earlier by the work of Schleiden and Schwann. Therefore, the concept of natural cell death, now known as apoptosis, is quite possibly one of the oldest ideas in cell biology. The first morphological description was provided in 1885 by Walther Flemming, whose elegant drawings show cell shrinkage, nuclear fragmentation and apoptotic body formation, all of which are now accepted hallmarks of apoptosis (BOX 1).

The study of apoptosis then drifted in and out of fashion for most of the next 100 years until, in 1951, Alfred Glucksman described

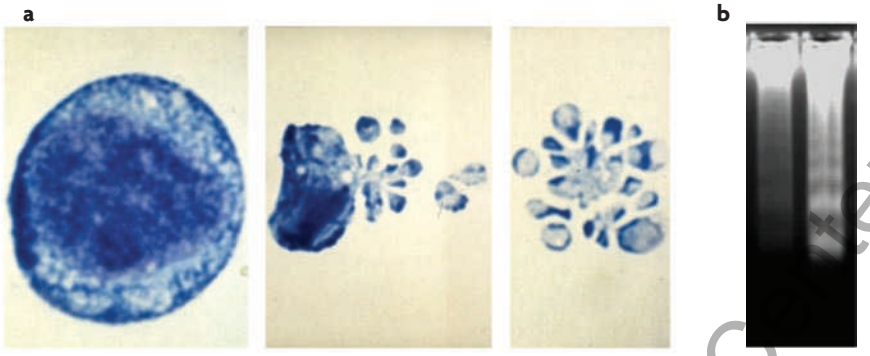


Box 1 | **Hallmarks of apoptosis**

Apoptosis is a tightly regulated multi-step pathway that is responsible for cell death not only during development, but also in adult multicellular organisms, in which it partly controls cell numbers. It is characterized by cell shrinkage, chromatin condensation, and nuclear and cell fragmentation. These features result in the formation of apoptotic bodies that are then engulfed by neighbouring phagocytic cells (see part **a** of the figure for images of a leukaemia cell and a cell undergoing classical apoptosis; note the condensed chromatin, the cell shrinkage and the fragmentation of the cell into apoptotic bodies).

Internucleosomal DNA cleavage yields a ladder pattern in electrophoretic gel, which is a biochemical hallmark of apoptosis (see part **b** of the figure for an image of a DNA electrophoretic gel with a lane from control cells on the left and a lane from cells undergoing apoptosis on the right).

Another hallmark of apoptosis is the movement of the membrane lipid phosphatidylserine from the inner to the outer side of the plasma membrane, where it then functions as a recognition signal for phagocytic cells to engulf apoptotic cells.



karyopyknosis as a distinct form of cell death, characterized by nuclear shrinkage and chromatin condensation[3]. John Saunders' and Richard Lockshin's work on the role of cell death during chick and insect wing development set the scene for the discovery that was subsequently to emerge from Alister Currie's laboratory in Aberdeen, Scotland[4,5]. Possibly influenced by these experiments, the Australian pathologist John Kerr began to describe an unusual form of cell death seen following ischaemic injury in liver tissue and coined the term shrinkage necrosis in the 1960s[6]. During sabbatical leave in Aberdeen, Kerr collaborated with Andrew Wyllie and Alister Currie. They characterized and defined this form of cell death and coined the term apoptosis. It was during this stay that Allison Crawford, a Ph.D. student, drew their attention to the literature on cell death in the developing embryo, including Gluckman's seminal review. History repeated itself, however, and little notice was taken of this elegant piece of observational work. It was only following Wyllie's publication in *Nature* in 1980 (REF. 7), which described the induction of thymocyte apoptosis associated with the activation of an endogenous endonuclease, that other scientists began to take real notice (TIMELINE). A subsequent review paper in the same year outlined the biological importance of apoptosis[8]. It took 18 years for the elusive endonuclease, DNA fragmentation factor subunit beta (DFFB; also known as CAD), to be cloned and characterized by Shige Nagata[9], but that is another story! Importantly, the formation of a characteristic DNA ladder gave us a biochemical marker for apoptosis, and scientists, initially in the field of immunology, began to explore the role and underlying mechanism of this phenomenon (BOX 1). At the time of writing there is hardly a field of modern biology that has not resulted in the publication of papers on some aspect of apoptosis. In 2002, Bob Horvitz shared the Nobel Prize in Physiology or Medicine for his pioneering work on unravelling some of the fundamental aspects of the biology of apoptosis, using *Caenorhabditis elegans* as a model system. His Nobel lecture provides a fascinating insight into his contribution to the field[10].

The concept of the undead cell. The idea that tumour development and progression could be influenced by apoptosis goes back to Kerr, Wyllie and Currie's original 1972 paper, in which they showed that the observed growth rate of tumours was less than the predicted rate as a result of a high level of endogenous tumour cell apoptosis. Subsequent studies showed that apoptosis played an integral part in tumour growth, progression and resistance to therapy, as reviewed by Kerr *et al.* in 1994 (REF. 11). The average adult human body generates approximately 60 billion cells per day, and as a consequence an equal number of cells must die by apoptosis to maintain cell homeostasis. Therefore, it is not surprising that deregulation of apoptosis can lead to an accumulation of undead cells and contribute to tumour development. What is really surprising is that the incidence of cancer is as low as it is when you think of the enormous number of cells that undergo apoptosis on a daily basis and the opportunity therein for malfunctions in the apoptotic process to occur. This fact has often been noted in seminars by Gerard Evan to illustrate how well-regulated apoptosis is. However, the regulation of apoptosis does become corrupted and tumours can develop[12–14] (FIG. 1). Once tumour biologists began to take the concept of the undead cell seriously, the mechanisms that regulate apoptosis began to emerge.

Bcl-2 proteins: gatekeepers of death

BCL-2 was the first protein involved in the regulation of apoptosis to be molecularly characterized. The *BCL2* gene was originally identified by Yoshide Tsujimoto[15] while he was working in Carlo Croce's laboratory. Croce was identifying genes in B cell lymphomas and naming them *BCL1* (for B cell lymphoma gene 1), *BCL2* and so on. The association between *BCL2* expression and translocation break points in B cell lymphomas suggested that *BCL2* might be an oncogene. *BCL2* was subsequently cloned by Tsujimoto[16] and independently by Mike Cleary[17]; both papers were published in the summer of 1986. In an interview with the journal *Cell Death & Differentiation* in 2004, David Vaux recalls receiving *BCL2* cDNA in early 1987 from Cleary and carrying out experiments to determine whether *BCL2* could function as an oncogene in a similar way to *BCR–ABL* in chronic myeloid leukaemia[18,19]. Using a retroviral transfection system, Vaux transfected *BCL2* into a myeloid cell line that was dependent on interleukin 3 (IL-3) for its survival. Similar to many groundbreaking experiments, disappointment had to be endured before the sweet smell of success. For example, when the BCL-2-transfected cells were transferred to soft agar, no clones grew, and clones would be expected if *BCL2* were acting as a true oncogene, such as *MYC*. Furthermore, when the *BCL2*-transfected cells were injected into syngeneic mice, no tumours formed. It really looked as though BCL-2 did not have oncogenic potential. However, when Vaux re-examined his agar plates he noticed that there was obvious cell death in the control plate where IL-3 had been removed, but little cell death in the

BCL2-transfected counterparts (this observation is reminiscent of Fleming's discovery of penicillin). In liquid culture, *BCL2*-transfected cells did not proliferate in the absence of IL-3 but, importantly, they also did not die; this was the experiment that demonstrated the ability of BCL-2 to control apoptosis. It is interesting to note that in this seminal 1988 *Nature* paper[20] there is no mention of the term apoptosis! In the same year, Yoshide Tsujimoto also showed that BCL-2 could protect against various cell death-inducing compounds[21]. Use of the term apoptosis in conjunction with *BCL2* was finally made in three papers published in the early 1990s[22–24]. A few short months after Vaux's 1988 paper[20] was published, John Reed and colleagues demonstrated that *BCL2* could function as a bona fide proto-oncogene. NIH3T3 fibroblasts stably transfected with a *BCL2* construct produced tumours in mice with a latency period of approximately 4 weeks. When these tumours were allowed to progress, they were lethal after approximately 3 months[25]. However, BCL-2 remained enigmatic because it did not transform cells in the same way as MYC or Ras; instead it was oncogenic owing to its capacity to enable cell survival in inappropriate conditions[26]. In 1990, Reed's laboratory[27] showed that suppression of BCL-2 expression using antisense RNAs made leukaemia cells susceptible to death by apoptosis. After the publication of these papers, there was an explosive output of research on the role of BCL-2 and its family members in the regulation of apoptosis and the contribution of this family to cancer development and resistance to treatment. Of course, the main thrust of this research was to try and understand how these proteins regulated apoptosis.

BCL-2 and the mitochondrion. It took years to get a clearer idea of how BCL-2 mediated its anti-apoptotic effect, and some aspects remain contentious today. The experiment that started the metaphorical ball rolling was carried out in 1990 by David Hockenbery[28], who was working in Stanley Korsmeyer's laboratory. Hockenbery showed that BCL-2 could suppress the production of reactive oxygen species by the mitochondrion, suggesting that the anti-apoptotic effects of BCL-2 were mediated at the level of this subcellular organelle. Then, in 1996 Liu *et al.*[29] showed that the release of cytochrome *c* from the mitochondrion was crucial for the execution of the apoptotic pathway because it activated caspase 9, which in turn activated caspase 3. Both of these caspases are crucial for the execution phase of apoptosis. This discovery was amazing given that cytochrome *c* was already extremely well known as a component of the mitochondrial respiration chain, and no one would have guessed its Jekyll and Hyde character. Depending on the situation, cytochrome *c* contributes to the life-sustaining role of the mitochondrion or acts as a driving force for death; there are few other proteins in biology with these types of opposing functions. The following year, Yang and colleagues showed that BCL-2 suppressed this release of cytochrome *c*[30] and therefore prevented caspase activation. For details of the roles of caspases and the mitochondrion in apoptosis, see excellent reviews by Creagh and Kroemer[31,32].

A growing family. During the 1990s, membership of the Bcl-2 family grew and the proteins discovered fell into one of three subfamilies. Proteins such as BCL-X_L (also known as BCL2L1)[33], BCL-W[34] and MCL1 (REF. 35) all contain three or four BCL-2 homology (BH) domains, which are required for their anti-apoptotic function. These domains drive interactions with other Bcl-2 family members, particularly at the level of the mitochondrion, where they regulate the release of pro-apoptotic mediators, such as SMAC (also known as DIABLO)[36,37]. The second subfamily consists of pro-apoptotic members, such as BAX[38] and BAK[39], which can form pores or interact with pore-forming proteins at the level of the mitochondrial membrane, a function that is antagonized by BCL-2. The discovery of a third Bcl-2 subfamily, which consists of proteins that have only a short BH3 domain, was important because these proteins provided a link between the terminal effector process and the signalling network that informs the cell of its status regarding events such as growth factor stimulation, redox balance, DNA integrity, cell attachment and microtubule function. The pro-apoptotic function of BAX is normally held in check by its interaction with BCL-2; however, the BH3-only protein BIM (also known as BCL2L11)[40] can antagonize this interaction and allow BAX to drive apoptosis. Members of the BH3-only subfamily include BID[41] and Bcl-2 antagonist of cell death (BAD)[42]. The activity of these proteins is normally kept in check by controlling their expression or by post-translational modification; for a review, see REF. 43.

Although BCL-2 was originally identified as a crucial factor in the development of B cell lymphomas, it also plays an important part in other tumours. In addition to activation of BCL-2 by chromosomal translocations in cancer, increased BCL-2 expression is found in other tumours, such as non-Hodgkin's lymphomas and certain lung cancers[44,45]. Since these discoveries, several other mechanisms have been identified that can lead to increased BCL-2 expression, including hypermethylation of *BCL2* and loss of microRNAs that normally downregulate BCL-2 expression[46,47]. For example, the transcription factor p53 is mutated in most human cancers, and some of its direct targets include BAX, BID and the BH3-only proteins PUMA (also known as BBC3) and NOXA (also known as PMAIP1), all of which are pro-apoptotic members of the Bcl-2 family[48–53]. Any impairment of p53 function leads to deregulation of apoptosis signalling pathways and increases tumorigenesis.

The use of knockout mouse models has contributed substantially to our understanding of the role of the ~20 members of the extended Bcl-2 family, in which redundancy has undoubtedly complicated the picture in relation to cancer development and treatment. Although *Bad*-knockout mice seem to develop normally, they go on to develop B cell lymphomas[54], and show increased rates of lymphoblastic leukaemia following sublethal γ-irradiation. *Bid*-knockout mice also develop a type of myelomonocytic leukaemia[55]. Haematopoietic precursors from these mice demonstrated increased myeloid colony formation, which may explain the pre-malignant hyperplasia observed. Finally, *Bim*-knockout mice also display a hyperplasia, which leads to increased tumour development on a tumour-prone background[56]. Despite these findings in mice we still have little information on the role of the Bcl-2 family in human cancer, although one might reasonably expect it to be similar to the role in mice given how closely the two species are related genetically.

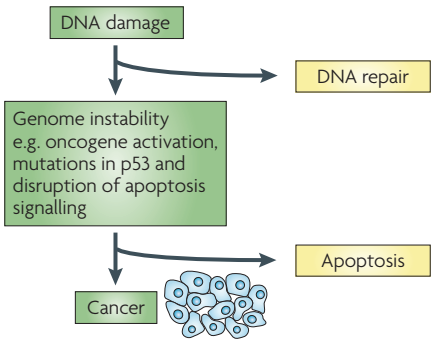


Figure 1 | **The pathway from DNA damage to cancer.** To cope with DNA damage, cells have evolved a sophisticated repair system. Failure of this system leads to genomic instability, which triggers apoptosis under normal physiological circumstances. Should mutations in key apoptosis signalling proteins and oncogene activation also occur, then tumour development is a likely scenario.

A human face. Several studies on the role of BCL-2 and BAX in human tumours have provided interesting results in terms of patient prognosis. In a subgroup of lymphomas, the level of BCL-2 expression correlates with a poor prognosis and response to therapeutic agents[57]. Overexpression of BCL-2 is common in various solid human tumours, including renal, stomach and brain cancer[58–60]. However, the prognostic importance depends on the tumour type, and in some cases there is no correlation with disease progression[61–63]. Low levels of BCL-2 correlate with poor prognosis in patients with breast cancer[64]. The picture is similarly unclear when BAX expression is examined in relation to tumour development and response to therapy. One might intuitively expect that a tumour with high levels of BAX would respond better to chemotherapy and have a better prognosis. Several studies in the mid 1990s fulfilled this molecular prophesy and showed that there was a positive correlation between expression of BAX and tumour response and prognosis[65,66]. Subsequent studies either supported these findings or found that there was no correlation or even a negative correlation with BAX expression[67–69]. Such findings probably result from the fact that the sensitivity of cancer cells to specific drugs can be regulated through the expression patterns of the BH3-only family members and not simply through the expression levels of BAX and BCL-2 (REF. 70).

BCL-2 as a therapeutic target. One of the key clinical problems in oncology is dealing with drug-resistant tumours. The mechanistic insights into the extended Bcl-2 family gained over the past two decades have contributed considerably to our understanding of the underlying biology of drug resistance and have highlighted ways of dealing with this problem. Since the original observations of Vaux showing that BCL-2 expression can induce cell survival, the role of BCL-2 in drug resistance has been slowly unravelled. In 1991, Charles Sentman and colleagues[71] showed that lymphocytes from *Bcl2*-knockout mice survived poorly, and this study together with Vaux's research undoubtedly influenced subsequent work on the role of BCL-2 in drug resistance. In 1992 and 1993, Miyashita[48] and Reed[49] showed that BCL-2 overexpression conferred resistance to various chemotherapeutic drugs used in cancer treatment. Such studies suggested for the first time that BCL-2 operated at a point in the death pathway downstream of the point at which these drugs normally trigger apoptosis. In other words, BCL-2 functions as a road block to drug-induced cell death. In 1997, Webb *et al.*[72] showed that antisense RNA knockdown of BCL-2 improved patient response in a Phase I clinical trial. Since these groundbreaking studies, the field has developed, and now there are several small molecule agonists and antagonists in various stages of preclinical development. Progress in this area of research has recently been reviewed by Lessene *et al.*[73] (FIG. 2).

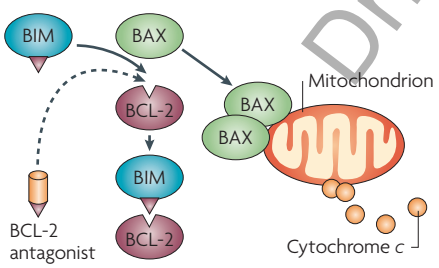


Figure 2 | **Controlling cytochrome *c* release.** This figure shows one scheme to explain how BH3-only proteins, such as BIM (also known as BCL2L11), can interact with BCL-2 to allow the release of BAX, which in turn drives cytochrome *c* release from the mitochondrion. This molecular understanding has formed the foundation for the design of small molecule BCL-2 antagonists, such as ABT-737.

Established tumour genes and apoptosis

While members of the Bcl-2 family were being identified, many other oncogenes and tumour suppressor genes were implicated in the regulation of apoptosis. To list all of these genes in this article and the contributions that they made to our understanding of the function of apoptosis in tumour biology is not feasible. Instead, I have chosen to focus on two of perhaps the most surprising and influential findings that involve the oncogene *MYC* and the tumour suppressor p53, which have an interlinked history in our understanding of how they regulate apoptosis and therefore contribute to tumour cell survival or death. Key experiments in the early 1990s (surely a golden period in apoptosis research) helped us understand the mechanisms by which these genes control apoptosis.

MYC: a key to life and cell death. By 1990, it was already well established that MYC can transform cells, and much of the published work focused on the relationship between MYC and cell proliferation. Then a rather remarkable counter-intuitive discovery was made independently by three laboratories. In 1991, John Cleveland[74] and colleagues showed that IL-3-dependent cells rapidly underwent apoptosis when deregulated MYC was expressed in the absence of IL-3. Subsequently, Doug Green[75] in San Diego, USA, and Gerard Evan[76] in London, England, showed that MYC could sensitize cells to undergo apoptosis in the absence of survival factors and that MYC induces a default apoptosis pathway. These studies showed the Janus-like nature of this oncogenic transcription factor. These seminal observations were also relevant for the mechanism is of action of other oncogenes. In a publication in 1990, Strasser *et al.*[77] showed that MYC and BCL-2 cooperated in the development of lymphoid tumours in transgenic mice, confirming the synergy between MYC and BCL-2 that had previously been observed *in vitro* by Vaux *et al.*[20]. The experiments of Green and Evan provided an elegant explanation of these observations — deregulated MYC induces a state of increased proliferation that is kept in check by a concomitant increase in apoptosis in normal tissues when the levels of growth or survival factors are limiting. Overexpression of BCL-2 blocks MYC-stimulated apoptosis, and clonal expansion of tumours that express MYC and BCL-2 can occur. This concept has been supported by the work of Soucie *et al.*[78] and de Alboran *et al.*[79], who showed that apoptosis is blocked in *Myc*-knockout cells. Why do some oncogenes, such as MYC, induce apoptosis under appropriate conditions? It is probably a molecular safety valve that exists to prevent tumour development. In other words, for MYC-driven tumorigenesis to occur, a forced increase in the expression of BCL-2 or another death-suppressing mechanism must also occur — a double hit. In the absence of this second hit, MYC normally suppresses anti-apoptotic proteins such as BCL-2 and BCL-X_L and allows a certain amount of tumour apoptosis to occur[80,81]. This in turn supports the evidence that MYC drives apoptosis by regulating BAX expression; Dansen and colleagues[82] used a switchable transgenic mouse model to show that when BAX was inactivated, MYC expression failed to drive apoptosis.

The use of animal models to study apoptosis and tumorigenesis has always been informative. Driven by the need to understand how the expression of specific genes in specific tissues contributed to tumour formation, innovative mouse models were developed, in particular models in which the expression or activity of specific oncogenes could be regulated. In 1999, two publications changed our concept of oncogene contribution to tumour formation and apoptosis. Tetracycline-regulated systems, such as those used by Dean Felsher and Michael Bishop[83], allowed *Myc* expression to be controlled in mouse tumour models. In the system developed in Gerard Evan's

laboratory[84], MYC activity was controlled by the administration of 4-hydroxytamoxifen. Thus, deregulated MYC can be used to induce tumour growth, and the kinetics of proliferation can be followed or MYC can be switched off to investigate tumour regression. In the case of mammary carcinomas, the tumour phenotype could be re-established by reactivating MYC[84]. However, when MYC expression was turned off in osteosarcomas, in addition to tumour regression, differentiation of the tumour cells occurred. Reactivation of MYC triggered apoptosis rather than the tumour growth observed in the mammary carcinoma model. Therefore, the cellular context in which MYC is expressed is relevant, and any MYC-based cancer therapies that may be developed in the future must recognize this aspect of MYC regulation. Despite many elegant experiments over the past 15 years, the detailed mechanism by which MYC induces apoptosis has remained unclear.

p53: the guardian of the genome. Although *MYC* can be considered the archetypal oncogene, *TP53* is without doubt its tumour suppressor equivalent; p53 also intimately regulates apoptosis, and the elucidation of how it does this has a timeline which parallels that of MYC. By the early 1990s, *TP53* was already recognized as a tumour suppressor gene and was known to be mutated in most human cancers. In 1991, Yonish-Rouach *et al.*[85] showed that wild-type expression of p53 in leukaemia cells induced apoptosis. This was followed by two back-to-back publications in 1993, which showed that thymocytes could undergo apoptosis in either a p53-dependent or a p53-independent manner in *Tp53*-knockout mouse models[80,81]. It is now known that a range of cytotoxic agents, including those used to treat cancer, can induce p53 expression (at least in cells in which the p53 system is working) and, consequently, apoptosis. Subsequent work in 1993 focused on elucidating the underlying biology by which p53 induces apoptosis in cells. As p53 is a transcription factor, it was natural to explore whether it regulated the expression of proteins that are known to be involved in the regulation of apoptosis, particularly members of the Bcl-2 family. For example, two publications in 1994 (REFS 48,86) and one in 1995 (REF. 49) showed that p53 regulated the expression of BAX in both *in vitro* and *in vivo* systems. In addition to controlling members of the Bcl-2 family, p53 controls the expression of other key apoptosis-regulating proteins[87] (FIG. 3).

The CD95 death receptor and cancer

A considerable amount of excitement was generated in the fledgling apoptosis community in 1989 as a result of a publication in *Science* by Peter Krammer and colleagues[88]. The idea of treating tumours with specific antibodies was of particular interest and Krammer's laboratory in Germany had produced a monoclonal antibody (APO1-specific) that induced apoptosis in lymphoid cells. Meanwhile, Shige Nagata and Shin Yonehara in Japan had immunized mice with the human fibroblast cell line FS-7 in an attempt to produce monoclonal antibodies against the interferon receptor. During the screening phase of this process, Yonehara accidentally found an antibody that was cytotoxic to the target cells. The antigen recognized by this antibody was called FAS (FS-7-associated surface antigen)[89] and within a year of starting, Nagata's laboratory[90] cloned and sequenced the *FAS* cDNA. In this seminal paper they also showed that apoptosis was the form of death induced when FAS-specific antibodies were added to FAS-expressing cells. In fact, the two laboratories had independently cloned the same molecule, which was renamed CD95.

The structure of CD95 suggested that it belonged to the tumour necrosis factor (TNF) receptor family and that there should also be a natural ligand that was similar to TNF. A fortuitous collaboration with Pierre Golstein in France enabled the CD95 ligand (CD95L) to be cloned and characterized, and the results were published in *Cell* in 1993 (REF. 91). CD95L was indeed a member of the TNF superfamily.

But what about Krammer's initial discovery, that CD95-specific antibodies induced the apoptosis of transformed lymphocytes that expressed CD95? To support this finding, the Krammer group[88] injected human-specific CD95 antibodies into tumour-bearing nude mice and saw a dramatic regression of the growing tumours. However, in 1992 Jun Ogasawara joined the Nagata laboratory and began to generate hybridoma cell lines that produced antibodies specific for the mouse CD95 antigen. To increase the production of the antibodies, he injected a hybridoma into the mouse peritoneal cavity and to his surprise found that within a few hours all the mice were dead owing to massive liver failure caused by antibody-induced hepatocyte destruction[92]. Such results stopped any thought of using CD95-specific antibodies

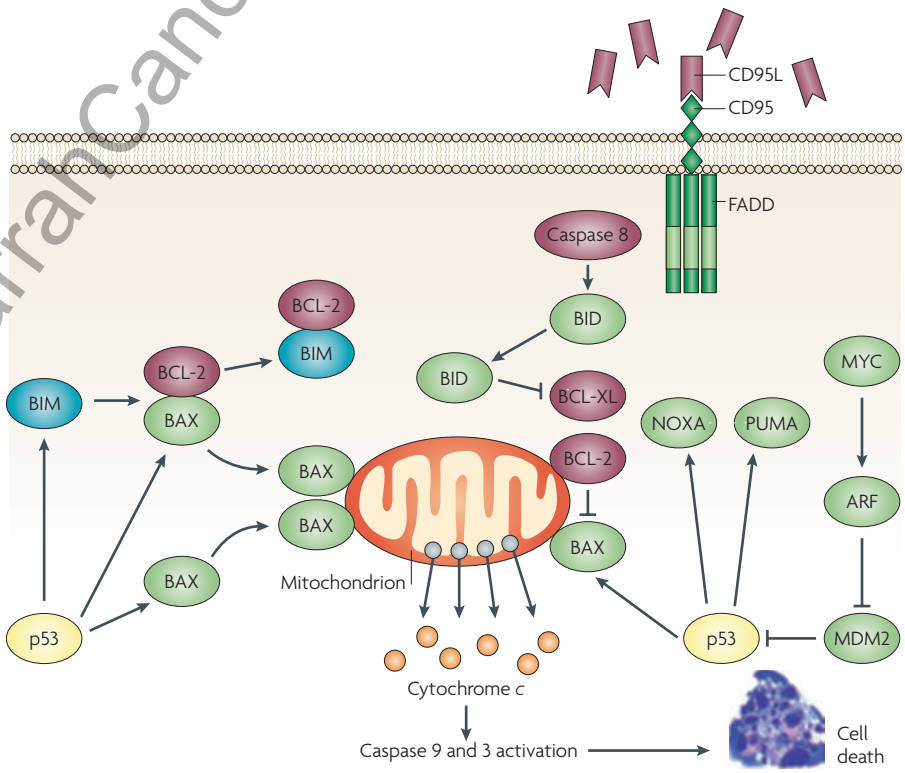


Figure 3 | **Signalling pathways in apoptosis.** The interactions between Bcl-2 family members, the CD95 (also known as FAS) system, MYC and p53 are shown. This is not a comprehensive signalling pathway diagram, but does give a sense of the intricate signalling involved in the regulation of apoptosis. FADD, FAS-associated death domain protein.

as a possible anticancer agent. The fact that CD95 is a member of the TNF receptor family enabled the identification of several other death receptors, including TRAIL (also known as TNFSF10) and its receptors TR10A (also known as TRAIL1) and TR10B (also known as TRAIL2). Fortuitously, antibodies that target these receptors and TRAIL do not induce marked liver failure in mice. Recombinant TRAIL and TRAIL receptor-specific antibodies are currently being tested in clinical trials[93–95].

Intriguingly, in the mid 1990s Krammer and colleagues[96] noticed that treating tumour cells with anticancer agents involves the CD95 system and leads to an increase in CD95 ligand expression[97]. Further evidence to support this concept came from work demonstrating that a wide range of tumour cell lines and tumour cells from patients expressed CD95L. In addition to CD95L, CD95 itself was upregulated in cells exposed to cytotoxic agents, especially when they expressed wild-type p53 (REFS 98,99). These studies also showed that the promoter of *CD95* contained p53 response elements, which explained its upregulation following drug-induced DNA damage.

Immune evasion. In the mid 1990s, the concept that tumour cells could fight back and kill the cells of the immune system that sought to control them gained considerable appeal, probably in part owing to the tumour counter-attack moniker given to this idea. The concept of tumours fighting off lymphocytes for their own survival had a Faustian attraction to it. The idea was based on the observation that tumour cells often had low levels of CD95 expression with concomitant high levels of CD95L expression. These tumour cells could therefore evade the immune system by using CD95L to engage CD95 on immune cells and provoke their destruction[99–102]. This idea was based on the finding that CD95L is expressed by cells in immune-privileged sites such as the eye, where corneal transplants are accepted without the need for immunosuppressive drugs. CD95L-expressing tumour cells induced apoptosis in CD95-expressing lymphocytes *in vitro,* and tumour-infiltrating lymphocytes underwent apoptosis in CD95L-expressing tumours in animal models[103]. However, work by Allison *et al.*[104] and others showed that tumour cells expressing CD95L are killed by infiltrating neutrophils. This debate, which originated in the mid 1990s, is still not convincingly settled.

The future

The successful treatment of cancer is one of the greatest challenges of modern medicine. However, this challenge has a devious, cunning and innovative foe in the tumour cell, a cell that has the survival instincts of the craftiest cat. Although we have made considerable strides in understanding how tumour cells survive and die over the past 20 years, current therapies still largely rely on a 'blunderbuss' drug treatment approach. However, there are encouraging signs that our understanding of the molecular mechanisms of apoptosis is enabling us to develop more rational approaches to cancer treatment. One promising approach is the design of antagonists to BCL-2, and considerable progress has been made in this area. Small molecular inhibitors that interfere with the interactions between Bcl-2 members, for example between BCL-2 and BAX, have been shown to promote apoptosis. Perhaps the most advanced of these compounds are the ABT-263 and ABT-737 molecules, which have good activity in animal models and are orally available[105]. These compounds (currently in Phase I and II clinical trials) have been shown to be active as single agents in several haematological and solid malignancies and also synergize with several other cytotoxic agents[70]. Of course, these therapeutic developments are based on the advances that we have made during the past 20 years in elucidating the underlying biology of apoptosis. It is also worth noting that apoptosis is no longer the only mechanism that suppresses tumour growth. As our understanding of apoptosis developed at the molecular level, so did our understanding of other forms of cell death. This is particularly true of autophagy and, arguably to a lesser extent, senescence. Why a cell needs several death mechanisms and how they are regulated remains a question that is currently unanswered and is certainly a challenge for the future. I am tempted in the final sentence to try and predict what the future holds, but looking at how the field of apoptosis has developed during the past 20 years with its many twists and turns, I might be best advised to keep such thoughts to myself.

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DATABASES

Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
MYC | *TP53*

UniProtKB: http://www.uniprot.org
BAD | BAK | BAX | BCL-2 | BCL-X_L | BID | CD95 | CD95L | DFFB | IL-3 | MCL1 | SMAC

FURTHER INFORMATION

Thomas G. Cotter's homepage: http://www.ucc.ie/ucc/depts/biochemistry/staff/tcotter.html

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