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Isolation of genes controlling apoptosis through their effects on cell survival

Research Article

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Abbreviations: Complementary DNA, (cDNA); Factor-dependent continuous cell line from the Paterson Institute, (FDCP-1); Interleukin-3, (IL-3); Phytohaemagglutinin, (PHA); Polymerase chain reaction, (PCR); Receptor for activated protein kinase C 1, (RACK1); Retroviral insertional mutagenesis, (RIM); Vacuolar ATPase, (vATPase); Walter and Elisa Hall Institute-105.726, (WEHI-105.726)

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Summary

The identification of the most suitable molecular targets for gene and drug therapy is the crucial first step in the development of new disease treatments. The rational identification of such targets depends on a detailed understanding of the pathological changes occuring at the molecular level. We have applied forward genetics approaches to the identification of the critical genes involved in the control of apoptosis in mammalian cells, since defective control of apoptosis underlies many diseases, including cancer and neurodegenerative diseases. We have identified two groups of genes by their effects on cell survival using retroviral cDNA functional expression cloning and retroviral insertional mutagenesis. The identification of these novel genes opens up new areas for apoptosis research and subsequently for the development of new gene and drug therapies.

I. Introduction

Apoptosis is now recognised as central to mammalian cell biology in general- no picture of any human or other mammalian system can be accepted as complete without some consideration of the potential role played by apoptosis. Apoptosis is consequently of profound significance in physiology, pathology and therapeutic medicine.

The analysis of the molecular mechanisms involved in apoptosis is therefore of great importance in developing gene and drug therapies for the many diseases where the control of apoptosis is perturbed. Apoptosis dysfunction occurs, for instance, in neurodegenerative diseases (too much apoptosis) and in autoimmune and neoplastic diseases (too little apoptosis) (Williams and Smith, 1993; Thompson, 1995; Hale et al, 1996;). Much cancer therapy, to quote an important example, relies on inducing apoptosis in tumour cells (Kerr et al, 1994). Since our knowledge of the molecular control of apoptosis is still incomplete, the identification of the genes involved in cell death and survival is of major importance in defining targets for rational design of gene and drug therapies.

The control of apoptosis is complex (e.g. Hengartner, 2000) and involves many genes. Some of these genes are now relatively well characterised, e.g. the bcl-2 family (Cory and Adams, 2002) and the caspase family (Thornberry and Lazebnik, 1998), but it is likely that many others have yet to be identified. Many strategies, such as isolation of proteins through their affinity for known components of the apoptosis machinery, are currently

being used to identify the missing molecules. We have chosen to identify genes controlling apoptosis and cell survival through the biological effects of the genes themselves on mammalian cells. This approach, sometimes known as forward genetics (Stark and Gudkov, 1999), is independent of previous knowledge and both can and does result in the identification of entirely novel and unpredicted components. This strategy also focuses automatically on those components which can actually affect the cell death/survival decision within the cell, as distinct from those associated with cell death but not having any controlling role. It is this first group which are likely to be of the greatest biological and clinical importance and which provide the best targets for gene and drug therapies.

Earlier work from Kimchi and co-workers using this sort of strategy resulted in the isolation of several important genes (Deiss et al, 1995; Cohen et al, 1997) including DAP-kinase, which can play an important role in metastasis (Inbal et al, 1997). Other groups have also used this approach, resulting in the isolation of several interesting and important genes (e.g. Hitoshi et al, 1998). We have used two related approaches within this general strategy; firstly, we have transfected cDNA libraries in expression vectors into clonal mammalian cells which are uniformly susceptible to apoptosis stimuli (**Figure 1**). The isolation and analysis of the cDNA clones expressed by cells which survive the stimulation of apoptosis identifies candidate apoptosis-controlling genes. The activity of these genes can later be confirmed by isolation of the sequence, re-cloning into an expression vector and expressing in fresh host cells which are then challenged with apoptosis stimuli. Secondly, we have infected clonal apoptosis-sensitive cell lines with retroviruses and again induced apoptosis under conditions where fewer than 1 in 10^4 host cells normally survive (**Figure 2**). In this case, the amplification of the host DNA flanking the inserted retrovirus using inverse PCR allows the identification of the gene affected by the insertion to produce the apoptosis-resistant phenotype. Our use of these two strategies resulted in the identification of several known apoptosis-controlling genes. The additional isolation of a larger number of genes not previously known to be involved in this process indicates that many more components of the cellular apoptosis-controlling machinery still remain to be identified.

II. Materials and methods

A. Cell culture

The W7.2 mouse thymoma cell line, originally derived from line WEHI-105.726 (Danielsen et al, 1983), and the FDCP1 haemopoietic cell line (Dexter et al, 1980), used as hosts were grown in RPMI 1640 with 10% fetal calf serum (Hyclone, UT, USA) at 37^{0}C in a 5% CO_2 humidified incubator. FDCP-1 medium was supplemented with mouse IL-3 (Dexter et al, 1980; McCarthy, 1993). Both cell lines were cloned in soft agar (McCarthy, 1993; Mourtada-Maarabouni et al, 2003) and apoptosis-sensitive clones containing less than 1 in 10^4 apoptosis-resistant cells were identified and grown to produce large stocks which were stored in liquid nitrogen. These target cells were used after thawing and a minimal number of subcultures in order to minimise the appearance of spontaneously apoptosis-resistant cells. The clones used in the present study were FDCP-1B, which had an even lower frequency of spontaneous IL-3 independence of 2(±1.9) x10^{-6}) (McCarthy, 1993), and W7.2c (Mourtada-Maarabouni et al, 2003).

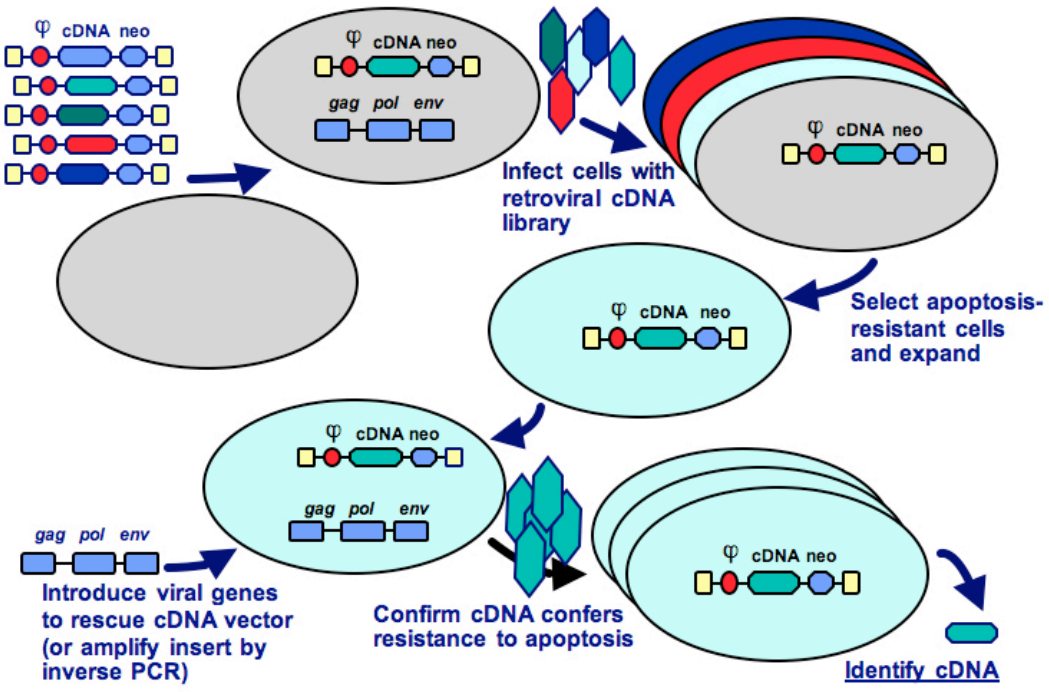

Figure 1. Production of apoptosis-deficient cell clones by retroviral cDNA library functional expression cloning.

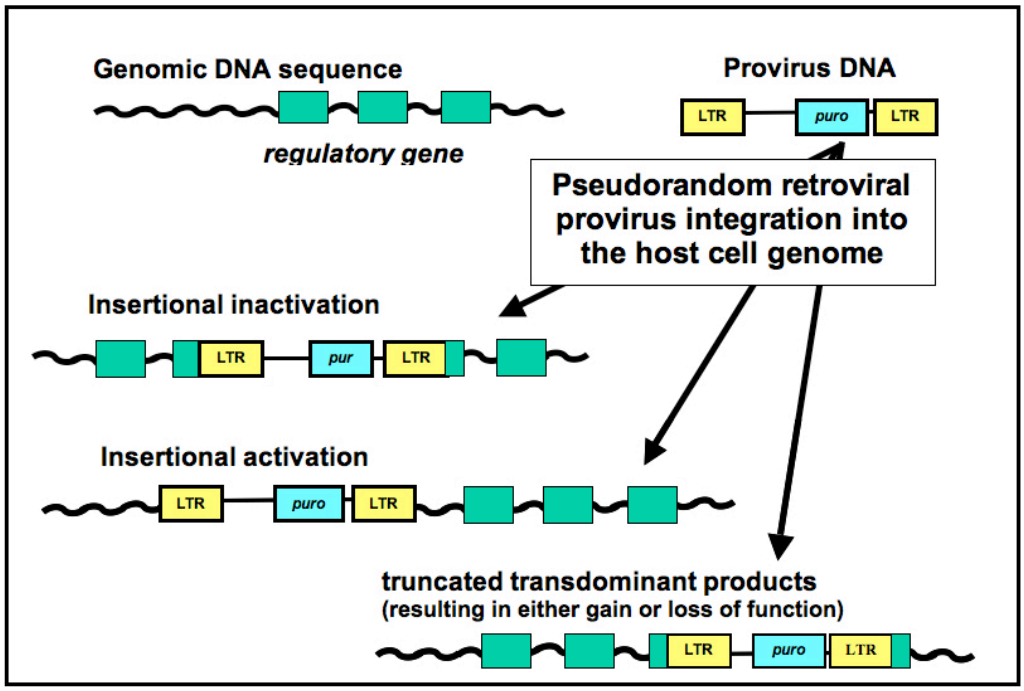

Figure 2. Different pathways for production of apoptosis-deficient cell clones by retroviral insertional mutagenesis (RIM).

B. cDNA functional expression cloning

Target cells were treated with 90 ng/ml tunicamycin at 3 x 10^5 cells/ml for 18 hours and washed before infection with retrovirus. Two different cDNA expression libraries were used, both in the pRUFneo retroviral expression vector (Rayner and Gonda 1994). cDNA for the first library was derived from human bone marrow stromal cells (Zannetino et al, 1996), and for the second library from FDCP1 cells (Rayner and Gonda, 1994)). ψ2 ecotropic murine packaging cells producing the libraries seeded at 5 x 10^6 cells per 225cm^2 flask were grown overnight to about 60% confluence and γ-irradiated using a ^{60}Co source (2500cGy). The supernatant was removed and 25ml of W7.2c cell suspension was added with 8μg/ml polybrene for 3 days co-culture. The cells in suspension were centrifuged and washed before being stored in aliquots in liquid nitrogen.

C. Selection of apoptosis-resistant clones

Several selection protocols were employed at different times to allow the identification of a range of different apoptosis-resistant mutant cells. Selection with γ-radiation was carried out using a ^{60}Co radiation source delivering a dose of 500-1000 cGy. Selection with dexamethasone (20- 50nM) was carried out for 6 days, after which time the cells were washed to remove the dexamethasone prior to cloning in soft agar (Mourtada-Maarabouni et al, 2003). Continuous treatment with dexamethasone during cloning was avoided since this would not allow the isolation of cells resistant to the apoptosis-inducing effects alone- any colonies growing in the continuous presence of dexamethasone would have to be resistant to its cytostatic effects as well.

Selection of W7.2c cells with Phytohaemagglutinin (PHA; HA16, Murex Biotech UK), either as the sole stimulus or after irradiation or dexamethasone treatment, was carried out by including the PHA in the soft agar cloning dishes at final concentrations of 5-10μg/ml.

cDNA inserts from apoptosis-resistant clones were amplified by PCR, using primers complementary to the adjoining vector, and sequenced (Mourtada-Maarabouni et al, 2003).

D. Retroviral Insertional Mutagenesis (RIM)

Target W7.2c and FDCP-1B cells were infected with the retroviral vector pBABEpuro (Morgenstern and Land, 1990) produced in the ecotropic packaging cell line GP+E86 (Miller and Miller, 1992). Target cells were pre-incubated with tunicamycin and washed before co-culture with gamma-irradiated packaging cells for 2 days in the presence of polybrene. After several infection cycles, infected W7.2c cells were washed and selected as above. Infected FDCP-1B cells were washed 3 x to remove IL-3 and incubated for 24 hours prior to cloning in soft agar; cells were incubated for a total of 7 days without IL-3, and IL-3 was then added to the soft agar. The site of retroviral insertion in surviving clones was determined by inverse PCR.

III. Results and Discussion

A. cDNA functional expression cloning

The use of techniques which depend on an unbiased screen based solely on the function of the gene has the potential to identify many genes which act by highly diverse mechanisms. This diversity is demonstrated in **Table 1** which lists 18 genes isolated from W7.2c cells which survived apoptotic stimuli. They are therefore candidate apoptosis-regulating genes. However it is likely that several will be false positives- e.g. isolated from cells fortuitously carrying genomic mutations giving resistance to apoptosis. For several of the clones however, apoptosis-suppressing activity has been confirmed by re-expressing the inserts in fresh host cells and challenging with apoptosis-inducing stimuli (e.g. Protein phosphatase 4, RACK1 and rFau (antisense to Fau).

The sequences isolated by cDNA functional expression cloning include full protein-coding sequences, such as Onzin/PLAC8, as well as partial coding sequences, such as Protein Phosphatase 4, and antisense sequences, such as rFau. These sequences can be assumed to modulate apoptosis in very different ways. PLAC8/ Onzin, for example, is likely to act as an inhibitor of apoptosis (Rogulski et al, 2005), whereas the partial mRNA sequence of Protein Phosphatase 4 identified appears to act by inhibiting the activity of endogenous pro-apoptotic Protein Phosphatase 4 (Mourtada-Maarabouni et al, 2003). The partial antisense sequence of Fau identified is likely to suppress apoptosis by hybridising to the mRNA of endogenous pro-apoptotic Fau (Mourtada-Maarabouni et al, 2004).

Table 1. Candidate apoptosis-regulating genes identified by cDNA functional expression cloning

Gene name(s)	cDNA library selected	Challenge for isolation	Host cell for isolation	cDNA originally isolated	Confirmation of effects on cell survival
PPP4C; Protein Phosphatase 4, catalytic subunit	Mouse FDCP1 cDNA in retroviral vector pRUFneo (Rayner and Gonda, 1994)	Dexamethasone followed by γ-radiation	W7.2c (Mourtada-Maarabouni et al, 2003)	Partial, sense	Yes (Mourtada-Maarabouni et al, 2003)
Fau; MNSFbeta	Mouse FDCP1 cDNA in retroviral vector pRUFneo (Rayner and Gonda, 1994)	Dexamethasone followed by γ-radiation	W7.2c (Mourtada-Maarabouni et al, 2003)	Partial, antisense	Yes (Mourtada-Maarabouni et al, 2004)
Gnb2l1/RACK1 ; Receptor for Active C Kinase 1	Mouse FDCP1 cDNA in retroviral vector pRUFneo (Rayner and Gonda, 1994)	γ-radiation followed by PHA	W7.2c (Mourtada-Maarabouni et al, 2003)	Partial, sense	Yes (Mourtada-Maarabouni et al, 2005)
Atp6v1e1; Vacuolar/ lysosomal ATPase, subunit E	Mouse FDCP1 cDNA in retroviral vector pRUFneo (Rayner and Gonda, 1994)	γ-radiation followed by PHA	W7.2c (Mourtada-Maarabouni et al, 2003)	Partial, sense	Yes (Anderson and Williams, 2003)
Gas5; Growth Arrest Specific transcript 5	Mouse FDCP1 cDNA in retroviral vector pRUFneo (Rayner and Gonda, 1994)	γ-radiation	W7.2c (Mourtada-Maarabouni et al, 2003)	Not applicable	-
Plac8; Onzin; C15	Mouse FDCP1 cDNA in retroviral vector pRUFneo (Rayner and Gonda, 1994)	γ-radiation followed by PHA	W7.2c (Mourtada-Maarabouni et al, 2003)	Full coding sequence, sense	Yes (Rogulski et al, 2005)
Limk2; LIM-motif-containing protein kinase 2	Mouse FDCP1 cDNA in retroviral vector pRUFneo (Rayner and Gonda, 1994)	Cloning in PHA	W7.2c (Mourtada-Maarabouni et al, 2003)	Partial, sense	-
Fus; Tls; Pigpen	Mouse FDCP1 cDNA in retroviral vector pRUFneo (Rayner and Gonda, 1994)	γ-radiation followed by PHA	W7.2c (Mourtada-Maarabouni et al, 2003)	Partial, sense	-
Ucp2; Uncoupling protein 2	Mouse FDCP1 cDNA in retroviral vector pRUFneo (Rayner and Gonda, 1994)	Dexamethasone followed by PHA	W7.2c (Mourtada-Maarabouni et al, 2003)	Partial, sense	-
Prtn3; mPR3; Proteinase 3	Mouse FDCP1 cDNA in retroviral vector pRUFneo (Rayner and Gonda, 1994)	Etoposide	W7.2c (Mourtada-Maarabouni et al, 2003)	Partial, sense	-
HTRA1; PRSS11; HTRA serine peptidase 1	Human bone marrow stromal cells (Zannettino et al, 1996)	PHA	W7.2c (Mourtada-Maarabouni et al, 2003)	Partial, sense	-

Gene name(s)	cDNA library selected	Challenge for isolation	Host cell for isolation	cDNA originally isolated	Confirmation of effects on cell survival
RPLP1; Ribosomal protein, large, P1	Human bone marrow stromal cells (Zannettino et al, 1996)	PHA	W7.2c (Mourtada-Maarabouni et al, 2003)	Full coding sequence, sense	-
TncRNA; Trophoblast-derived noncoding RNA	Human bone marrow stromal cells (Zannettino et al, 1996)	PHA	W7.2c (Mourtada-Maarabouni et al, 2003)	Not applicable	-
S100A6; S100 calcium binding protein A6 (calcyclin)	Human bone marrow stromal cells (Zannettino et al, 1996)	PHA	W7.2c (Mourtada-Maarabouni et al, 2003)	Partial, sense	-
SEC61A1; HSEC61; protein transport protein SEC61 alpha subunit	Human bone marrow stromal cells (Zannettino et al, 1996)	PHA	W7.2c (Mourtada-Maarabouni et al, 2003)	Partial, sense	-
HNRPD; AUF1A; Heterogeneous nuclear ribonucleoprotein D; AU-rich element RNA binding protein 1	Human bone marrow stromal cells (Zannettino et al, 1996)	PHA	W7.2c (Mourtada-Maarabouni et al, 2003)	Partial, sense	-
TNFAIP8; GG2-1, SCC-S2, MDC-3.13; Tumor necrosis factor, alpha-induced protein 8	Human bone marrow stromal cells (Zannettino et al, 1996)	PHA	W7.2c (Mourtada-Maarabouni et al, 2003)	Partial, sense	-
GMFB; Glia maturation factor beta	Human bone marrow stromal cells (Zannettino et al, 1996)	PHA	W7.2c (Mourtada-Maarabouni et al, 2003)	Partial, sense	-
CTSD; CPSD; Cathepsin D	Human bone marrow stromal cells (Zannettino et al, 1996)	PHA	W7.2c (Mourtada-Maarabouni et al, 2003)	Partial, sense	Yes, e.g. Liaudet-Coopman et al, 2006

In other cases, mutated/truncated proteins may be produced which can have either dominant negative inhibitory activity, or which may be constitutively activated.

The anti-apoptotic effect of the partial sense protein phosphatase 4 sequence was confirmed by isolation of the cDNA insert from the genomic DNA of the corresponding W7.2c clone using PCR and subsequent expression in fresh host cells (Mourtada-Maarabouni et al, 2003). Many of the proteins important in the control of apoptosis are regulated by phosphorylation and dephosphorylation, e.g. the Bcl-2 family of apoptosis regulators (e.g. Deng et al, 1998; Chiang et al, 2001). The identification of Protein Phosphatase 4 as functionally important in apoptosis suggests that it may act on one or more of these. The sequence antisense to Fau is also of particular significance since a Fau antisense sequence is also found in the Finkel-Biskis-Reilly murine sarcoma virus (Finkel et al, 1976). The anti-apoptotic effect of Fau antisense sequences, and the pro-apoptotic effects of Fau, have been confirmed directly (Mourtada-Maarabouni et al, 2004). Fau may therefore act as a tumour suppressor, and down-regulation of Fau may be important in oncogenesis.

One of the cDNA sequences isolated by sequential selection with γ-radiation and PHA is a partial cDNA for the receptor for activated protein kinase C (RACK1),

which includes the 3'-untranslated sequence of the mRNA. Although this sequence does not contain the full coding sequence of RACK1, it up-regulates endogenous RACK1, presumably by interacting with endogenous regulatory molecules. Studies on the expression of full length RACK1 have confirmed its anti-apoptotic activity, which may be related to its established interactions with Src kinases (Chang et al, 2002), integrins (Liliental and Chang, 1998) or other molecules (Mourtada-Maarabouni et al, 2005).

Vacuolar ATPase subunit E was identified in two separate screens. Firstly, by temporary withdrawal of IL-3 from BAF-3 IL-3-dependent cells (Anderson and Williams, 2003) and, independently, by selection of W7.2c cells with γ-radiation followed by PHA. In both cases the suppression of apoptosis appeared to be due to indirect effects on the endogenous vATPase through regulatory molecules which modulate the activity of the vATPase. This proton pump can affect both cytoplasmic and vacuolar/lysosomal pH, as well as other aspects of cell metabolism (reviewed by Nishi and Forgac, 2002).

B. Retroviral Insertional Mutagenesis (RIM)

The information which has flowed from the human and mouse genome projects over the past few years has been very valuable in allowing the rapid identification of the sites of retroviral insertion in cells showing resistance to apoptosis (**Table 2**). This has made it possible to identify the flanking sequences obtained by inverse PCR (e.g. Nowrouzi et al, 2006; Shin et al, 2004) and so to suggest the identity of novel candidate apoptosis-regulating genes. Two of the genes identified by RIM have been shown to be involved in the control of apoptosis. Firstly, Notch1 has been shown to play a crucial role in the control of cell fate, including the control of apoptosis (e.g. Jundt et al, 2002). Secondly, the insulin-like growth factor receptor (Igf1r) has been shown to regulate apoptosis and to play an important role in oncogenesis in many tissues (e.g. Roschier et al, 2001). Spink2, on the other hand, could not be demonstrated to play any significant role in apoptosis in the Jurkat human T-cell line, or in the TF-1 human growth factor dependent cell line (Hedge and Williams, unpublished work). This serves as a reminder that the candidate apoptosis-regulating genes listed in both **Table 1** and **Table 2** are bound to include some false positives. Further studies are required in each case to confirm or refute the potential roles in apoptosis control.

In the present paper we have confirmed that forward genetics, either using cDNA functional expression cloning or using RIM, is a very valuable strategy for the analysis of the molecular controls on apoptosis. In several cases, entirely unpredicted genes have been identified, each of which opens up a new avenue for apoptosis research. Since regulation of apoptosis is crucial to many diseases, this molecular dissection of apoptosis identifies novel targets for the gene and drug therapy of these diseases.

Acknowledgments

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Table 2. Candidate apoptosis-regulating genes identified by retroviral insertional mutagenesis

Mouse chromosome insertion	Gene closest to insertion	Gene sequence associated with insertion	Confirmation of effects on cell survival
10	Cdh23; Cadherin-23; Otocadherin (insertion into intron)	GI:24475914	-
3	Gstm1; Glutathione-S-Transferase Mu-1 (insertion into intron)	GI: 68051724	-
2	Notch1	GI :31543331	Yes, e.g. Jundt et al, 2002
14	Pheromone receptor V3R6	GI:26083204	-
5	Spink2; Serine peptidase inhibitor, Kazal type 2	GI:34304086	-
7	Igf1r; insulin-like growth factor I receptor	GI:3025893	Yes, e.g. Roschier et al, 2001

References

Anderson C L and Williams **G T (2003)** Apoptosis gene hunting using retroviral expression cloning. **SciWorld J** 3, 51-58.

Chang B Y, Harte R A, Cartwright C A **(2002)** RACK1: a novel substrate for the Src protein-tyrosine kinase. **Oncogene** 21, 7619-7629.

Chiang C W, Harris G, Ellig C, Masters S C, Subramanian R, Shenolikar S, Wadzinski B E, Yang E **(2001)** Protein phosphatase 2A activates the proapoptotic function of BAD in interleukin-3-dependent lymphoid cells by a mechanism requiring 14-3-3 dissociation. **Blood** 97, 1289-1297.

Cohen O, Feinstein E, Kimchi A **(1997)** DAP-kinase is a Ca2+ calmodulin-dependent cytoskeletal-associated protein kinase with cell death-inducing functions that depend on its catalytic activity. **EMBO J** 16, 998-1008.

Cory S, Adams J M **(2002)** The BCL2 family: Regulators of the cellular life-or-death switch. **Nat Rev Cancer** 2, 647-656.

Danielsen M, Peterson D O, Stallcup M R **(1983)** Immunological selection of variant mouse lymphoid cells with altered glucocorticoid responsiveness. **Mol Cell Biol** 3, 1310-1316.

Deiss L P, Feinstein E, Berissi H, Cohen O, Kimchi A **(1995)** Identification of a novel serine threonine kinase and a novel 15-kd protein as potential mediators of the gamma-interferon-induced cell-death. **Genes Dev** 9, 15-30.

Deng X, Ito T, Carr B, Mumby M, Stratford May Jr M **(1998)** Reversible phosphorylation of Bcl2 following interleukin-3 or bryostatin 1 is mediated by direct interaction with PP2A. **J Biol Chem** 273, 34157-34163.

Dexter T M, Garland J, Scott D, Scolnick E, Metcalf D **(1980)** Growth of factor-dependent hematopoietic precursor cell-lines. **J Exp Med** 152. 1036-1047.

Finkel M P, Reilly Jr C A, Biskis B O **(1976) Recent Results Cancer Res** 54, 92-103.

Hale A J, Smith C A, Sutherland L C, Stoneman V E A, Longthorne V L, Culhane A C, Williams G T **(1996)** Apoptosis: Molecular regulation of cell death. **Eur J Biochem** 236, 1-26.

Hengartner M O **(2000)** The biochemistry of apoptosis. **Nature** 407, 770-776.

Hitoshi Y, Lorens J, Kitada S I, Fisher J, LaBarge M, Ring H Z, Francke U, Reed J C, Kinoshita S, Nolan G P (1998) Toso, a cell surface specific regulator of Fas-induced apoptosis in T cells. **Immunity** 8, 461-471.

Inbal B, Cohen O, PolakCharcon S, Kopolovic J, Vadai E, Eisenbach L, Kimchi A **(1997)** DAP kinase links the control of apoptosis to metastasis. **Nature** 390, 180-184.

Jundt F, Anagnostopoulos I, Forster R, Mathas S, Stein H, Dorken B **(2002)** Activated Notch1 signaling promotes tumor cell proliferation and survival in Hodgkin and anaplastic large cell lymphoma. **Blood** 99, 3398-3403.

Kerr J F R, Winterford C M, Harmon B V **(1994)** Apoptosis - its significance in cancer and cancer-therapy. **Cancer** 73, 2013-2026.

Liaudet-Coopman E, Beaujouin M, Derocq D, Garcia M, Glondu-Lassis M, Laurent-Matha V, Prebois C, Rochefort H, Vignon F **(2006)** Cathepsin D: newly discovered functions of a long-standing aspartic protease in cancer and apoptosis. **Cancer Letts** 237, 167-179.

Liliental J, Chang DD **(1998)** Rack1 a receptor for activated protein kinase C interacts with integrin beta subunit. **J Biol Chem** 273, 2379-2383.

McCarthy N J, **(1993)** Apoptosis induced by cancer chemotherapeutic drugs and its genetic suppression. D. Phil (University of Birmingham).

Miller D G and Miller A D **(1992)** Tunicamycin treatment of CHO cells abrogates multiple blocks to retrovirus infection one of which is due to a secreted inhibitor. **J Virol** 66, 78-84.

Morgenstern J P, Land H **(1990)** Advanced mammalian gene-transfer - high titer retroviral vectors with multiple-drug selection markers and a complementary helper-free packaging cell-line **Nucl Acid Res** 18, 3587-3596.

Mourtada-Maarabouni M, Kirkham L, Farzaneh F, Williams G T **(2004)** Regulation of apoptosis by fau revealed by functional expression cloning and antisense expression. **Oncogene** 23, 9419-9426.

Mourtada-Maarabouni M, Kirkham L, Farzaneh F, Williams G T, **(2005)** Functional expression cloning reveals a central role for the receptor for activated protein kinase C 1 (RACK1) in T cell apoptosis. **J Leuk Biol** 78, 503-514.

Mourtada-Maarabouni M, Kirkham L, Jenkins B, Rayner J, Gonda T J, Starr R, Trayner I, Farzaneh F, Williams G T **(2003)** Functional expression cloning reveals proapoptotic role for protein phosphatase 4. **Cell Death Diff** 10, 1016-1024.

Nishi T, Forgac M **(2002)** The vacuolar (H+)-ATPases - Nature's most versatile proton pumps. **Nat Rev Mol Cell Biol** 3, 94-103.

Nowrouzi A, Dittrich M, Klanke C, Heinkelein M, Rammling M, Dandekar T, von Kalle C, Rethwilm A **(2006)** Genome-wide mapping of foamy virus vector integrations into a human cell line. **J Gen Virol** 87, 1339-1347.

Rayner J R, Gonda T J **(1994)** A simple and efficient procedure for generating stable expression libraries by cDNA cloning in a retroviral vector. **Mol Cell Biol** 14, 880-887.

Rogulski K, Li Y J, Rothermund K, Pu L X, Watkins S, Yi F H, Prochownik E V **(2005)** Onzin a c-Myc-repressed target promotes survival and transformation by modulating the Akt-Mdm2-p53 pathway. **Oncogene** 24, 7524-7541

Roschier M, Kuusisto E, Suuronen T, Korhonen P, Kyrylenko S, Salminen A **(2001)** Insulin-like growth factor binding protein 5 and type-1 insulin-like growth factor receptor are differentially regulated during apoptosis in cerebellar granule cells. **J Neurochem** 76, 11-20.

Shin M S, Fredrickson T N, Hartley J W, Suzuki T, Agaki K, and Morse H C **(2004)** High-throughput retroviral tagging for identification of genes involved in initiation and progression of mouse splenic marginal zone lymphomas. **Cancer Res** 64, 4419-4427.

Stark G R, Gudkov A V (1999) Forward genetics in mammalian cells: functional approaches to gene discovery. **Hum Mol Genet** 8, 1925-1938.

Thompson C B **(1995)** Apoptosis in the pathogenesis and treatment of disease. **Science** 267, 1456-1462.

Thornberry N A, Lazebnik Y **(1998)** Caspases: Enemies within. **Science** 281, 1312-1316.

Williams G T, Smith C A **(1993)** Molecular regulation of apoptosis - genetic controls on cell-death. **Cell** 74, 777-779.

Zannettino A C W, Rayner J R, Ashman L K, Gonda T J, Simmons P J **(1996)** A powerful new technique for isolating genes encoding cell surface antigens using retroviral expression cloning. **J Immunol** 156, 611-620.