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Understanding cell cycle and cell death regulation provides novel weapons against human diseases

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Abstract. Wiman KG, Zhivotovsky B (Cancer Center Karolinska (CCK), Stockholm; Karolinska Institutet, Stockholm, Sweden; Lomonosov Moscow State University, Moscow, Russia). Understanding cell cycle and cell death regulation provides novel weapons against human diseases (Review Symposium). *J Intern Med* 2017; **281**: 483–495.

Cell division, cell differentiation and cell death are the three principal physiological processes that regulate tissue homoeostasis in multicellular organisms. The growth and survival of cells as well as the integrity of the genome are regulated by a complex network of pathways, in which cell cycle checkpoints, DNA repair and programmed cell death have critical roles. Disruption of genomic integrity and impaired regulation of cell death may both lead to uncontrolled cell growth. Compromised cell death can also favour genomic instability. It is becoming increasingly clear that dysregulation of cell cycle and cell death processes plays an important role in the development of major disorders such as cancer, cardiovascular disease, infection, inflammation and neurodegenerative diseases. Research achievements in these fields have led to the development of novel approaches for treatment of various conditions associated with abnormalities in the regulation of cell cycle progression or cell death. A better understanding of how cellular life-and-death processes are regulated is essential for this development. To highlight these important advances, the Third Nobel Conference entitled 'The Cell Cycle and Cell Death in Disease' was organized at Karolinska Institutet in 2016. In this review we will summarize current understanding of cell cycle progression and cell death and discuss some of the recent advances in therapeutic applications in pathological conditions such as cancer, neurological disorders and inflammation.

Keywords: cell cycle, cell death, disease, therapy.

Introduction

Approximately 2500 years ago, the Chinese philosopher Confucius (551–479 BC) stated: 'While you do not know life, how can you know death?' However, since 1972, when the term 'apoptosis' was introduced [1], the rapid development of the cell death field has provided knowledge that helps us not only understand the molecular mechanisms of different types of cell death, but also recognize the phenomenon of cell death as an equally important physiological process as proliferation and differentiation. The cell cycle control machinery must ensure flawless duplication of the genome and cell division, which is the basis for self-replication. During the last few decades, major advances have been made in terms of understanding the mechanisms of cell cycle regulation at the molecular level. All three physiological processes (cell death, proliferation and differentiation) can efficiently regulate tissue homoeostasis in multicellular organisms. Cell growth and survival as well as genome integrity are regulated by numerous pathways that involve cell cycle checkpoints, programmed cell death and DNA repair. Uncontrolled cell growth can result from loss of genome integrity and dysregulation of cell death. Genomic instability can lead to accumulation of mutations in, or changes in expression levels of, cell death regulators. On the other hand, compromised cell death can favour genomic instability. Key regulators such as p53 may play important roles in the control of both cell death pathways and genomic instability, allowing a tight link between these processes. It is

important to note that in response to oncogenic stress or genotoxic insult, cells activate multiple factors that inhibit cyclin-dependent kinases (CDKs), the drivers of the cell cycle, and thereby prevent cell cycle progression, promote DNA repair or, if the damage cannot be repaired, eliminate the damaged and therefore potentially dangerous cell by programmed cell death.

Three Nobel Prizes in Physiology or Medicine have highlighted achievements in these fields of research. In 2001, Leland H. Hartwell, R. Timothy (Tim) Hunt and Paul M. Nurse were awarded the prize for their discoveries of key regulators of the cell cycle; in 2002, Sydney Brenner, H. Robert Horvitz and John E. Sulston received the prize for discoveries concerning genetic regulation of organ development and programmed cell death; and in 2016, Yoshinori Ohsumi was awarded the prize for his discoveries of mechanisms for autophagy.

Two types of cell death, apoptosis and necrosis, were known in the early 1970s; however, we now recognize more than 10 different modes of programmed cell death [2]. The Nomenclature Committee on Cell Death suggested several criteria for the classification of cell death [3], starting with morphology, going through biochemical manifestations and, finally, explaining the difference between essential and accessory aspects of regulated cell death. It should be noted that similar mechanisms could be attributed to embryonic development, adult tissue homoeostasis and cell death induced by various biological, chemical and physical agents.

The importance of dysregulated cell cycle progression and cell death in the pathogenesis of major diseases, such as cancer, ischaemia/reperfusion injury, atherosclerosis, infection, inflammation and neurological disorders, is now well established. Both physiological processes are essential for regulation of autoimmunity. Likewise, recent results indicate an interesting relationship between dysregulated cell death, cellular senescence and premature ageing. Here, we will summarize current understanding of cell cycle progression and cell death and discuss some of the recent advances in therapeutic applications in pathological conditions such as cancer, neurological disorders and inflammation.

Cell cycle progression, genome integrity and disease pathogenesis

The cell is driven forwards in the cell cycle by specific proteins termed cyclins and CDKs. There are four main types of cyclins in mammalian cells, cyclins A, B, D and E, that form various complexes with CDKs such as CDK1, CDK2, CDK4 and CDK6 (reviewed by Malumbres and Barbacid [4]). These complexes are responsible for progression of the cell through the cell cycle phases G1, S, G2 and M. Cell cycle progression is further regulated by two classes of cell cycle inhibitors: the INK4 proteins including p16 (INK4a) and p15 (INK4b) and the Cip/Kip family including p21, p27 and p57. These different inhibitors can block specific cyclin–CDK complexes and thus halt the cell cycle at specific points [5, 6].

A critical and therefore tightly regulated step is the transition from G1 to S. Once the cell has entered S phase, it is bound to continue through S, G2 and M and thus give rise to two daughter cells. According to the classical model, pRB, the product of the retinoblastoma (*RB1*) gene and prototype tumour suppressor, inhibits S phase entry by forming a complex with E2F1–3, three proteins that belong to the E2F transcription factor family (reviewed by Weinberg [7]). E2F1–3 can activate transcription of genes required for DNA synthesis, thus driving the cell through S phase to G2. Mitogenic signalling via cell surface receptors activates cyclin D1, D2 and/or D3 that form complexes with CDK4 or CDK6 during the G1 phase of the cell cycle. This leads to pRB phosphorylation by G1 cyclin–CDK complex, for example cyclin D1–CDK4, which releases E2F from RB and triggers S phase entry. More recent studies have indicated that cyclins, CDKs and E2Fs may also have additional cell cycle-independent functions [6, 8].

Another well-known tumour suppressor, p53, which is encoded by the *TP53* gene, is also part of the cell cycle control machinery. p53 is a transcription factor that binds specifically to DNA and regulates target genes involved in various cellular processes. It is expressed at low levels under normal conditions in the absence of cellular stress. p53 is stabilized in response to DNA damage, oncogenic stress and various other stress conditions and activates transcription of a number of genes that induce cell cycle arrest or apoptosis. In addition, p53 can regulate genes involved in various other processes, such as metabolism, and induce the cell cycle inhibitor p21, leading to G1 cell cycle arrest. p53 can also regulate the cell cycle inhibitors GADD45 and 14-3-3sigma; thus, its activation can efficiently block cell cycle progression. p53 is also a prominent cell death regulator, as discussed further below.

Two of the classical hallmarks of cancer cells are self-sufficiency in growth signals and insensitivity to antigrowth signals [9, 10]. At the molecular level, these two hallmarks can be linked to aberrant proliferation due to activation of classical oncogenes, for example Myc and Ras, and disruption of pRB-mediated regulation of G1 to S transition. It is conceivable that all tumours carry at least one genetic alteration that abrogates pRB function, directly or indirectly. Such alterations may include loss or inactivation of *RB1* itself, which is observed in 100% of retinoblastomas and bone and soft tissue sarcomas in retinoblastoma families. *RB1* inactivation occurs at variable frequencies in a wide range of other tumour types, for example small-cell lung cancer (SCLC) [11] and bladder cancer [12]. Alternatively, overexpression of D cyclins or CDK4 or CDK6, which can occur as a result of for instance chromosomal translocations (cyclin D1) or gene amplification (*CDK4*), may result in constitutive pRB phosphorylation and release of E2Fs. As a consequence, the normal control of S phase entry and cell division is abrogated. Yet another possibility is loss or inactivation of the cell cycle inhibitor p16, encoded by the *INK4a* (*CDKN2A*) gene, which normally controls CDK4 and CDK6 activity. Indeed, the *INK4a*/*CDKN2A* tumour suppressor gene is frequently inactivated by homozygous deletion or other mechanisms in human tumours [13].

Many human tumours carry inactivating mutations in *TP53* [14]. The great majority of these mutations are missense mutations that disrupt the ability of p53 to bind specifically to DNA and activate transcription, resulting in loss of cell cycle regulation via p53 (reviewed by Soussi and Wiman [14]). p53 is activated by oncogenic stress, that is aberrant DNA replication in cells in which growth is fuelled by activated oncogenes and disrupted cell cycle control. This curbs tumour development by induction of cell cycle arrest, senescence and/or apoptosis. The DNA damage response to aberrant cell cycle progression thus acts as an important barrier against tumour development [15, 16]. However, selection for *TP53* mutations or mutation of other genes in the DNA damage response pathway, for example *ATM* or *Chk2*, may lead to the emergence of clones that escape p53-dependent cell cycle arrest and/or cell death, and eventually tumour relapse. Thus, aberrations in the machinery that regulates cell cycle progression can give rise to various types of cancer.

Cell death mechanisms and pathological disorders

The best studied mode of cell death is apoptosis, which can be regulated via extrinsic or intrinsic pathways. Apoptosis is often associated with activation of caspases, a family of cysteine-dependent aspartate proteases, but can also be caspase independent, occurring even in the presence of efficient caspase inhibitors and displaying at least some morphological signs of apoptosis (e.g. partial chromatin condensation). Both pathways are activated upon formation of high-molecular weight protein complexes (DISC, with caspase-8 as the most upstream-located protease; apoptosome complex, with caspase-9 as the most upstream-activated enzyme; and PIDDosome, p53-inducible death domain-containing complex, with caspase-2 as the first activated enzyme) [17]. One of the most specific features of apoptosis is the elimination of dead cells by phagocytosis, which rescues tissues from inflammation.

Autophagy is characterized by cytosolic accumulation of autophagosomes. In contrast to apoptotic cells, whose clearance is regulated by phagocytosis, cells that die by autophagy have little or no association with phagocytes. A unique feature of autophagy is the degradation and recycling of cellular components. There are several forms of autophagy. Macroautophagy is characterized by formation of cytoplasmic double-membrane vesicles known as autophagosomes. These vesicles fuse with lysosomes where degradation and recycling of intracellular constituents occur. Formation and regulation of autophagosomes requires the accumulation of various proteins in high-molecular weight complexes [18]. Microautophagy and chaperone-mediated autophagy are two other types of autophagy.

The discovery of necroptosis, a novel and distinct form of cell death [19], indicated that necrosis can occur in a programmed manner. Plasma membrane permeabilization is tightly regulated during necroptosis, in contrast to necrosis. Necroptosis is initiated by formation of a high-molecular weight complex termed the necrosome. This complex activates the pronecroptotic protein MLKL resulting in the formation of the bilipid membranes of organelles and the plasma membrane essential for the expulsion of cellular contents, into the extracellular space, known as damage-associated molecular patterns (DAMPs). Of note, necroptotic cells are cleared from the immune system by a

pinocytosis mechanism, which is completely different from removal of apoptotic cells via phagocytosis [20].

Unlike apoptosis, the recently discovered cell death modality pyroptosis is a highly inflammatory form of programmed cell death. Pyroptosis is activated in response to infection with intracellular pathogens and is likely to form part of the antimicrobial response. In this type of cell death, a high-molecular weight complex termed the pyroptosome (also known as the inflammasome) which includes caspase-1 is formed. Pyroptosis and necroptosis both result in rupture of the plasma membrane and release of DAMP molecules into the extracellular milieu. Released cytokines recruit more immune cells and further perpetuate the inflammatory cascade in the tissue. Interestingly, macrophages undergoing pyroptosis are characterized by morphological features that are typical of apoptosis as well as necrosis [21].

Most recently, another form of regulated cell death, ferroptosis, was described. Ferroptosis is iron and ROS dependent and completely distinct from other forms of cell death at the morphological, biochemical and genetic levels. Regulation of ferroptosis is tightly linked to iron metabolism and lipid peroxidation [22].

Accumulated evidence suggests crosstalk between various cell death modalities (Fig. 1). Moreover, in many cases, inhibition of one cell death mechanism leads to a switch to another. Thus, inactivation of caspases or a significant decrease in the level of ATP can lead to a shift from apoptosis to

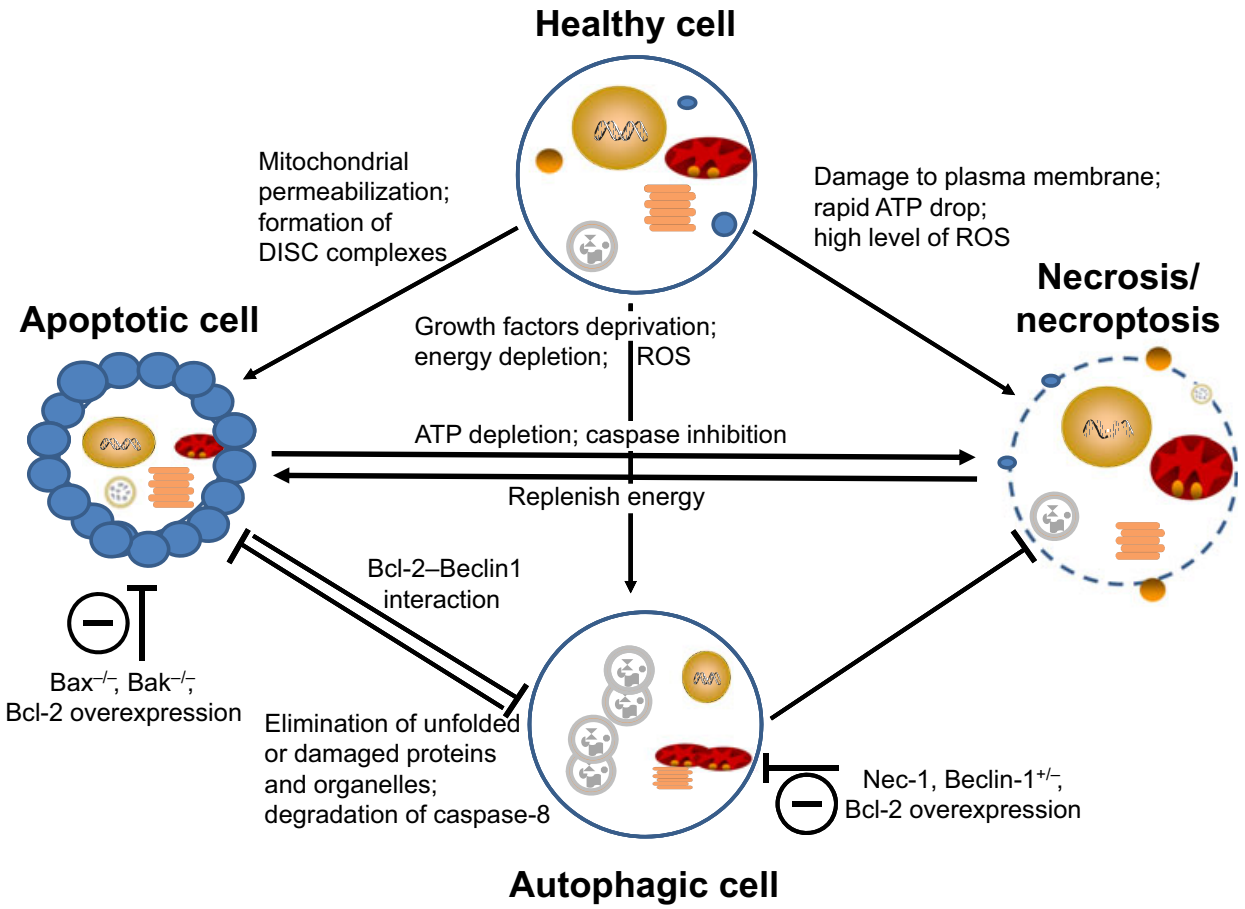

Fig. 1 *Crosstalk between various cell death modalities. In response to various type of damage, different cell death mechanisms can be activated. Moreover, crosstalk exists between the different forms of cell death. ROS, reactive oxygen species; DISC, death-inducible signalling complex.*

necrosis, or to a mixture of these two types of cell death. There are many examples of the same protein being involved in regulation of different types of cell death. Thus, p53, calpain and many other proteins are important for apoptosis and autophagy, and caspase-8 participates in the formation of high-molecular weight complexes essential for activation of apoptosis and necroptosis.

Different localization of the same protein might influence the activation of different modes of cell death, as exemplified by the key cell death regulator p53. Nuclear p53 activates transcription of pro-apoptotic genes, for instance *Puma*, *Noxa* and *Fas*, as well as cell cycle-arresting genes, such as *p21*, and can also promote autophagy. On the other hand, p53 localized in the cytoplasm may induce apoptosis by translocating to mitochondria and also inhibit autophagy. The exact molecular mechanism(s) is not fully understood.

Serine/threonine kinases RIP1 and RIP3 are involved in the regulation of both apoptosis and necroptosis. Bmf, which belongs to the BH3-only Bcl-2 family, is required for death receptor-induced necroptosis. Beclin 1, which was first identified as a Bcl-2-interacting protein, is involved in autophagy and can be inhibited by anti-apoptotic proteins Bcl-2 and Bcl-XL. Importantly, Beclin 1 possesses a BH3-only domain. Although all BH3-only proteins of the Bcl-2 family are known to induce apoptosis, Beclin 1 fails to do so; rather, it protects against various apoptosis triggers by stimulating autophagy [23]. Moreover, in response to growth factor withdrawal, caspase-mediated cleavage of Beclin 1 inactivates autophagy and promotes apoptosis by releasing pro-apoptotic factors from mitochondria. In this case, a caspase-generated fragment of Beclin 1 acts as an amplifier of apoptosis. Apparently, Bcl-2 family proteins are important for the regulation of most forms of programmed cell death.

The crosstalk between organelles and pathways can result in different forms of cell death, depending on both the nature and intensity of the stimulus and the type of cells. Also, suppression of the function of a particular intracellular compartment might switch one mode of cell death to another. Thus, significant lowering of ATP can shift apoptosis to necrosis. Likewise, inhibition of caspase activity can switch cell death from apoptosis to necrosis or autophagy, and activation of calpain-mediated cleavage of autophagy-regulated protein Atg-5 can lead to apoptosis rather than autophagic cell death [24]. Thus, investigation of the molecular mechanisms regulating various types of cell death and their crosstalk is vital, as they play a critical role in numerous biological processes.

Disturbances in various types of cell death have been implicated in a number of physiological and pathological processes. The consequence of too much cell death because of the presence of few cells in the population is loss of function characterized by pathophysiological features of neurodegenerative (Alzheimer's, Huntington's and Parkinson's diseases), haematological (aplastic and Fanconi anaemias, myelodysplastic syndrome) and autoimmune disorders (fulminant hepatitis, Hashimoto's thyroiditis, insulin-dependent diabetes mellitus, rheumatoid arthritis) and various conditions associated with bacterial and viral infections (AIDS, *Helicobacter pylori*, sepsis). On the other hand, too little cell death is associated with accumulation of many cells in the population, some of which may be the wrong type of cells. The best-known examples of this are various malignant diseases, autoimmune (autoimmune lymphoproliferative syndrome types I and II, systemic lupus erythematosus) and haematological disorders (polycythaemia vera, familial haemophagocytic lymphohistiocytosis) and diseases associated with viral infections (adenoviruses, baculoviruses, herpesviruses).

Treatment approaches

Cancer

Recent advances in the understanding of cell cycle and cell death have revealed disturbances at the molecular level in these pathways in various diseases, which could be potential targets for improved therapy. This is particularly clear for cancer. In fact, knowledge about cell cycle regulation as presented in the classical model above has led to a number of opportunities for novel cancer therapy. A few examples will be discussed here (see also Table 1).

Given the key role of CDKs for driving cell cycle progression and the probably universal inactivation of the pRB pathway, CDKs have appeared as attractive therapeutic targets. Several inhibitors of CDK4 and CDK6 are now being tested in the clinic (reviewed by Sherr *et al.* [13]). The CDK4 inhibitor palbociclib showed significantly increased progression-free survival in combination with the aromatase inhibitor letrozole in ER-positive,

Table 1 *Examples of drugs approved for cancer therapy by the US Food and Drug Administration during the last 3 years*

Year	Name	Origin	Target	Activity
2014	Idelalisib (Zydelig)	Small molecule	Inhibitor of PI3K delta; expressed primarily in blood cell lineages	Relapsed CLL in combination with rituximab Relapsed follicular B-cell non-Hodgkin lymphoma and small lymphocytic lymphoma in patients who have received at least two prior systemic therapies
	Ibrutinib (Imbruvica)	Antibody	Selective inhibitor of *Btk*, a gene that is disrupted in the human disease XLA. BTK is a signalling molecule of the BCR and cytokine receptor pathways	CLL
	Ceritinib (Zykadia)	Chemical compound	Inhibitor of ALK. *ALK* is a key gene implicated in the development of some lung cancers	ALK-positive metastatic NSCLC that has progressed on or is intolerant to crizotinib
	Nivolumab (OPDIVO)	Antibody	Anti-PD-1 antibody. EGFR-mutant inhibition	Advanced melanoma, advanced NSCLC, advanced RCC, classical Hodgkin lymphoma and advanced squamous cell carcinoma of the head and neck
2015	Alectinib (Alecensa)	Chemical compound	Kinase inhibitor that targets ALK and RET	ALK-positive, metastatic NSCLC which has progressed on or is intolerant to crizotinib

Table 1 (*Continued*)

Year	Name	Origin	Target	Activity
	Cobimetinib (Cotellic)	Chemical compound	Kinase inhibitor	Unresectable or metastatic melanoma with a *BRAF* V600E or V600K mutation, in combination with vemurafenib
	Palbociclib (Ibrance)	Chemical compound	CDK inhibitor	Combination with letrozole for the treatment of postmenopausal women with ER-positive, HER2-negative advanced breast cancer as initial endocrine-based therapy for metastatic disease. This indication is approved under accelerated approval based on PFS. Continued approval for this indication may be contingent upon verification and description of clinical benefit in a confirmatory trial
	Lenvatinib (Lenvima)	Chemical compound	RTK inhibitor that inhibits the kinase activities of VEGFR1, as well as other RTKs that have been implicated in pathogenic angiogenesis, tumour growth and cancer progression in addition to their normal cellular functions	Locally recurrent or metastatic, progressive, radioactive iodine-refractory differentiated thyroid cancer
	Sonidegib (Odomzo)	Chemical compound	Hedgehog pathway inhibitor	Adult patients with locally advanced BCC that has recurred following surgery or radiation therapy, or patients who are not candidates for surgery or radiation therapy
	Osimertinib (Tagrisso)	Chemical compound	EGFR-TKI, a targeted cancer therapy designed to inhibit both the activating, sensitizing mutations (EGFRm) and T790M, a genetic mutation responsible for EGFR-TKI treatment resistance	Metastatic EGFR T790M mutation-positive NSCLC patients who have progressed on or after EGFR-TKI therapy
2016	Venetoclax (ABT199; Venclexta)	Chemical compound	Bcl-2 inhibitor, an anti-apoptotic protein	CLL patients with 17p deletion who have received at least one prior therapy
	Cabozantinib (Cabometyx)	Chemical compound	Kinase inhibitor	Advanced RCC patients who have received prior anti-angiogenic therapy

Table 1 (*Continued*)

Year	Name	Origin	Target	Activity
	Lenvatinib (Lenvima)	Chemical compound	RTK inhibitor that inhibits the kinase activities of VEGFR1 (FLT1), VEGFR2 (KDR) and VEGFR3 (FLT4)	Combination with everolimus for the treatment of patients with advanced RCC following one prior anti-angiogenic therapy
	Atezolizumab (TECENTRIQ)	Antibody	Blocks PD-L1	For patients with advanced urothelial carcinoma or metastatic NSCLC and urothelial carcinoma. In combination with entinostat for triple-negative breast cancer
			Pembrolizumab (KEYTRUDA)	Antibody
	Blocks a PD-L1. A checkpoint inhibitor for use in all patients with PD-L1 and no EGFR or ALK genomic tumour aberrations	NSCLC with no prior systemic chemotherapy treatment		

PI3K, phosphoinositide-3 kinase; CLL, chronic lymphocytic leukaemia; Btk, Bruton's tyrosine kinase; XLA, X-linked agammaglobulinaemia; BCR, B-cell receptor; ALK, anaplastic lymphoma kinase; NSCLC, non-small-cell lung cancer; PD-1, programmed death receptor-1; RCC, renal cell carcinoma; CDK, cyclin-dependent kinase; ER, oestrogen receptor; HER2, human epidermal growth factor receptor 2; PFS, progression-free survival; RTK, receptor tyrosine kinase; VEGFR, vascular endothelial growth factor receptor; BCC, basal cell carcinoma; EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor; PD-L1, programmed death ligand 1. The main part of this information was obtained from clinicaltrials.gov.

HER2-negative breast cancer [25]. Palbociclib was approved by the US Food and Drug Administration (FDA) in 2015 for treatment of ER+/HER2- breast cancer in combination with letrozole as first-line therapy [26]. The drug has also been tested or is currently being tested in several clinical trials in other types of tumours, such as lung cancer [non-small-cell lung cancer (NSCLC)], liposarcoma and mantle-cell lymphoma. The novel CDK4/6 inhibitors ribiciclib and abemaciclib have similar target specificity as palbociclib but show differences in toxicity profile. Abemaciclib has higher monotherapy response rates and less haematological toxicity than palbociclib and ribiciclib, perhaps due to higher selectivity for CDK4 than for CDK6 [27].

Efforts are also under way to restore activity to p53 in human tumours. One approach is to block complex formation between p53 and its inhibitor Mdm2 in tumours that carry wild-type *TP53*. The prototype p53-Mdm2 inhibitor is Nutlin, and a number of novel compounds with a similar mechanism of action have been designed. Inhibition of p53-Mdm2 binding prevents Mdm2-mediated p53 degradation in the proteasome and thus enhances p53 protein levels, which will trigger cell cycle arrest and/or apoptotic cell death. Several p53-Mdm2 inhibitors including RG7112 and MI-773 have been or are being tested in clinical trials (for reviews, see [28, 29]). RG7112 has been tested in liposarcoma and leukaemias with clinical responses in a fraction of patients. Adverse events, mostly haematological, have been observed [30, 31].

A different strategy is restoration of p53 function by small molecules that promote correct folding of missense mutant p53. Such molecules, including PK-5174, SCH529074, NSC3197266 (ZMC1), PRIMA-1 and APR-246, have been identified by various strategies (reviewed by Bykov and Wiman [32]). A common theme among these molecules is the promotion of proper folding of mutant p53. The compounds PRIMA-1 (APR-017) and APR-246 are converted to methylene quinuclidinone (MQ), a Michael acceptor that binds covalently to cysteine residues in the mutant p53 core domain [33, 34]. MQ also inhibits thioredoxin reductase (TRxR1) [35] and depletes cellular glutathione [36, 37]. APR-246 showed relatively minor toxicity in a first-in-human phase I/IIa clinical trial in patients with haematological or prostate tumours. Clinical effects were observed in two patients [38]. Currently, APR-246 is being tested in a phase Ib/II trial in high-grade serous ovarian cancer in combination with standard chemotherapy. An alternative approach is based on restoration of the activity of p53 family member p73 in tumour cells by preventing its interaction with mutant p53 and inhibition of the N-terminally truncated Δp73 isoform that is a dominant negative inhibitor of p73 and p53 [39, 40].

Several types of tumours are associated with overexpression of Bcl-2 family anti-apoptotic proteins, such as Bcl-2, Bcl-XL or Mcl-1, leading to inhibition of intrinsic apoptosis. Moreover, in tumours that carry mutant p53, transcriptional activation of pro-apoptotic Bcl-2 family proteins Puma and Noxa is abrogated, leading to increased therapy resistance. A recent study demonstrated that the BH3 mimetics ABT-737, ABT-293 and ABT-199 were able to markedly improve tumour response to different anticancer treatments, highlighting Bcl-2 family proteins as crucial targets [41]. On 11 April 2016, the FDA approved venetoclax (ABT-199), a highly selective oral small-molecule Bcl-2 inhibitor for use in chronic lymphocytic leukaemia (CLL) patients who carry 17p deletion (deletion on the short arm of chromosome 17) and who have received at least one prior therapy. Moreover, patients with acute myeloid leukaemia (AML; relapsed/refractory or unfit for intensive chemotherapy) with a tolerable safety profile in a phase II study were more sensitive to venetoclax. Clinical and preclinical findings provide a convincing basis for evaluating the combination of venetoclax with other known agents in AML.

Navitoclax (ABT-263) is a potent and selective inhibitor of Bcl-W [42]. In an ongoing phase IIa study including patients with recurrent and progressive SCLC after at least one prior therapy, navitoclax has shown moderate single-agent activity in advanced and recurrent SCLC. However, preclinical models suggest that this compound can increase sensitivity of SCLC and other tumours to standard chemotherapy. Unfortunately, navitoclax inhibits Bcl-XL, causing reduced platelet lifespan and thrombocytopenia, which limits its clinical use. The current focus is combination therapy with navitoclax.

Recently, S63845, a compound that binds with high affinity to Mcl1, was developed [43]. The data obtained demonstrate a killing effect of S63845 in Mcl1-dependent cancer cells, including multiple myeloma, leukaemia and lymphoma cells. *In vivo*, S63845 shows potent antitumour activity as a single agent. These results support specific targeting of Mcl1 for the treatment of various tumours.

Thus, several successful attempts were made to specifically target the Bcl-2 family of anti-apoptotic proteins for the effective treatment of various haematological and solid tumours.

Programmed cell death protein 1 (PD-1) is encoded by the *PDCD1* gene in humans. PD-1 acts as an immune checkpoint and plays a vital role in down-regulating the immune system by preventing T-cell activation. PD-1 simultaneously promotes apoptosis in antigen-specific T cells in lymph nodes and reduces apoptosis in regulatory T cells [44]. Recently, FDA approved the first anti-PDL1 cancer immunotherapy for patients with a specific type of bladder cancer. Atezolizumab (TECENTRIQ™, Roche) was suggested for patients with locally advanced or metastatic urothelial carcinoma who are resistant to platinum-based chemotherapy. Another anti-PD-1 antibody, nivolumab (Opdivo, Bristol Myers Squibb), produced complete or partial responses in NSCLC, melanoma and renal cell cancer and was also approved by the FDA. Unfortunately, colon and pancreatic cancer patients did not respond to this drug. Pembrolizumab (Keytruda, Merck), which also targets PD-1 receptors, was approved by the FDA to treat metastatic melanoma. It has shown significant efficacy with few side effects.

The Smac protein, upon release from mitochondria, abrogates the activity of inhibitors of apoptosis proteases and makes tumour cells more sensitive to treatment. Recently developed Smac mimetics have demonstrated good antitumour activity. Amongst those that have entered the clinic, the most advanced is TL32711 (birinapant, APExBIO). Preclinical studies in patient-derived tumour xenotransplant models in mice have shown tumour regression induced by monotherapy with TL32711 as well as synergy when combined with other chemotherapeutic drugs. Patients with advanced solid tumours and lymphoma responded well to intravenous delivery of TL32711 without dose-limiting toxicities. Importantly, levels of cIAPs in tumours remained low during the first week after injection of TL32711. At present, a study of the effect of TL32711 in combination with five different chemotherapeutic drugs is continuing. Another bivalent Smac mimetic, HGS1029 (Human Genome Sciences), is in preclinical studies and has shown good antitumour activity in a number of tumour types alone and in combination with other agents. A high-affinity monovalent Smac mimetic, LCL161 (Novartis), is in clinical development as both a single agent and in combination with other drugs and has shown high antitumour activity in solid tumours. In the early 1990s, a member of the TNF superfamily, the TNF-related apoptosis-inducing ligand (TRAIL, also known as Apo2L), was shown to retain the unique ability to activate apoptosis selectively in cancer cells *in vitro* and *in vivo*. Since that time, several attempts have been made to develop TRAIL receptor agonists, some of which have demonstrated strong anticancer activity in preclinical studies [44]. Unfortunately, clinical trials testing the TRAIL receptor agonists revealed rather limited therapeutic benefit. In addition to a high level of hepatotoxicity, most primary cancer cells did not respond to TRAIL monotherapy. However, following the emergence of new data showing the additional activation of apoptotic pathways by TRAIL in cancer cells, novel revised TRAIL-based therapeutic concepts have been introduced into the cancer clinic. Based on this, several TRAIL-sensitizing agents were developed to overcome TRAIL resistance of cancer cells. These agents can be used in combination treatment of selected tumours. Thus, conatumumab, a fully human monoclonal antibody against TRAIL receptor 2, was suggested for combination treatment with birinapant in patients with relapsed ovarian cancer. This combination strategy is supported by several preclinical studies showing that Smac mimetics represent powerful sensitizers for TRAIL-induced apoptosis in a variety of cancers.

Neurological disorders

Accumulating evidence suggests the involvement of caspase-6 in axon degeneration and neurodegenerative disorders, such as Huntington's and Alzheimer's diseases. It was shown that caspase-6-mediated cleavage in mutated huntingtin protein is essential for the development of the specific behavioural and neuropathological features of Huntington's disease. Activation of caspase-6 was detected at early stages of Alzheimer's disease, and the activity of this enzyme was associated with the pathological lesions of the disease [45]. Recently, several therapeutic approaches based on modulation of caspase-6 activity were suggested for the treatment of neurodegenerative diseases; however, their therapeutic success is unclear.

In addition to caspase-6, the involvement of caspase-2 was shown to be important for cleavage of tau protein, which impairs cognitive and synaptic function in cellular and animal models of tauopathies. Experiments with tau mutants that cannot

be cleaved by caspase-2 showed prevention of infiltration of tau in spines, dislocation of glutamate receptors and impairment of synaptic function in cultured neurons, as well as prevention of memory deficits and neurodegeneration in mice. Moreover, downregulation of caspase-2 led to restoration of long-term memory in mice that had existing deficits [46]. A treatment strategy that includes inhibition of caspase-2 function for restoration of synaptic function and memory in patients with Alzheimer's disease via prevention of tau accumulation in dendritic spines was recently suggested. It is known that crushing the optic nerve leads to activation of apoptosis in retinal ganglion cells and downregulation of caspase-2, which is predominantly activated in retinal ganglion cells, inhibits this process. A combination of delivery of short interfering caspase-2 siRNA and inhibition of caspase-6 activated astrocytes and Müller cells, increased ciliary neurotrophic factor (CNTF) levels in the retina and led to enhanced regeneration of retinal ganglion cell axon. This study offers the possibility for development of novel therapeutic approaches for stimulating retinal ganglion cell survival and axon regeneration.

Work from Joseph's group revealed activation of caspase-8 and caspase-3/caspase-7, known executioners of apoptotic cell death that regulate microglia function [47]. Of note, this activation does not trigger cell death *in vitro* or *in vivo*. These caspases are activated in microglia in the ventral mesencephalon in individuals with Parkinson's disease and in the frontal cortex of those with Alzheimer's disease. Additional studies from the same group have shown that cIAP2-mediated regulation of caspase-3 controls the switch between pro-inflammatory activation and cell death in microglia. Targeting of cIAP2 by Smac mimetics, such as BV6, reduced the pro-inflammatory activation of microglial cells and promoted their death. These results indicate cIAP2 as a potential therapeutic target for modulation of pro-inflammatory activation of microglia and neurotoxicity detected in neurodegenerative disorders.

Inflammation-associated disorders

Inflammasome activation is crucial for the host defence against pathogens. Recent studies have shown an important role of inflammasomes in the pathogenesis of certain inflammatory disorders such as inflammatory bowel disease, rheumatoid arthritis and atherosclerosis. Crohn's disease, also known as regional enteritis, is a type of inflammatory bowel disease that affects the gastrointestinal tract from mouth to anus, causing a wide variety of symptoms. SNPs in the inflammasome NLRP3 promoter were shown to be associated with increased susceptibility to Crohn's disease in humans [48]. These polymorphisms affected NLRP3 expression and IL-1β production in cells stimulated with Toll-like receptor (TLR) agonists. The recently developed drug infliximab (Remicade, Johnson & Johnson) was shown to be effective for the treatment of Crohn's disease, particularly in patients who did not respond to other types of treatment. Unfortunately, infliximab can only be used for acute, short-term treatment because it interferes with TNF-α activity, which can predispose patients to serious infections.

Gout (also known as podagra) is sterile inflammatory arthritis caused by monosodium urate (MSU) crystal deposition in various tissues, which is characterized by formation of red, tender, hot, swollen joints. MSU crystals can induce specific activation of the NLRP3 inflammasome, both *in vitro* and *in vivo* [49]. It is exciting that preliminary clinical trials of anakinra or rilonacept have shown efficient IL-1β blockade in gout patients, and the absence of adverse effects. It will be interesting to determine whether long-acting therapies such as canakinumab are able to abolish chronic gout flares over time.

Recent studies have shown that autophagy links inflammasomes to atherosclerotic progression [50]. This process increases the accumulation of cholesterol crystals, which may lead to a cycle of atherosclerotic propagation in the setting of autophagy deficiency. It is well known that mammalian target of rapamycin (mTOR) inhibitors or TLR7 ligands can intensify basal autophagy in macrophages. Therefore, these drugs were tested as potential plaque-stabilizing compounds. Indeed, delivery of the mTOR inhibitor everolimus promoted a stable plaque phenotype, whereas local administration of the TLR7 ligand imiquimod stimulated inflammation and plaque progression. Hence, more drugs capable of inducing autophagy should be tested in plaque-derived macrophages to investigate the feasibility of this strategy.

Concluding remarks

It is now clear that our vision from more than 20 years ago that 'modulation of apoptosis may

perhaps be employed more generally as a therapeutic principle in the future' [51] can be expressed more broadly to relate to multiple cell death modalities. The progress in cell cycle and cell death research has been crucial not only for improving understanding of the pathogenesis of various diseases associated with disturbances of these two fundamental biological processes, but also as a basis for the development of novel strategies for their efficient treatment. After many years of studies of basic mechanisms in many laboratories, we are now seeing the exciting and dynamic development of various novel therapeutic strategies that target key components of the cell cycle and cell death machineries. A number of novel compounds have been tested in clinical studies, and some have already been approved for clinical use. We can expect further progress in the coming years and ultimately greatly improved treatment of common disorders such as cancer and inflammation-associated and neurological diseases.

Conflict of interest statement

KGW is a cofounder and shareholder of Aprea Therapeutics AB, a company that develops p53-based cancer therapy including APR-246. KGW is also a member of its Clinical Advisory Board.

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