Pathol. Oncol. Res. (2011) 17:791–800
DOI 10.1007/s12253-011-9433-4

REVIEW

Necroptosis: Biochemical, Physiological and Pathological Aspects

Zsuzsanna Dunai · Pal I. Bauer · Rudolf Mihalik

Received: 6 April 2011 / Accepted: 24 June 2011 / Published online: 21 July 2011

Abstract Programmed cell death is a key component of tissue homeostasis, normal development and wide variety of diseases. Conventional view refers to programmed cell death form as caspase-mediated apoptosis while necrosis is considered as an accidental and unwanted cell demise, carried out in a non-regulated manner and caused by extreme conditions. However, accumulating evidences indicate that necrotic cell death can also be a regulated process. The term necroptosis has been introduced to describe a cell death receptor-induced, caspase-independent, highly regulated type of programmed cell death process with morphological resemblance of necrosis. Necroptosis recently has been found to contribute to a wide range of pathologic cell death forms including ischemic brain injury, neurodegenerative diseases and viral infection, therefore a better understanding of the necroptotic signaling machinery has clinical relevance.

Keywords Necrosis · Necroptosis · Programmed cell death · RIPK1 · RIPK3 · TNF · Death receptor

Abbreviations

PCD	Programmed cell death
LC3	Microtubule-associated protein light chain 3
CICD	Caspase-independent cell death
FasL	(also known CD95L) Tumor necrosis factor ligand superfamily member 6
Fas receptor	(also known CD95 receptor) Tumor necrosis factor receptor superfamily member 6
TNFα	Tumor necrosis factor-alpha, tumor necrosis factor
RIPK1	(also known RIP1) Receptor-interacting protein kinase 1, receptor-interacting serine/threonine-protein kinase 1
TRAIL	(also known Apo-2L) Tumor necrosis factor-related apoptosis-inducing ligand, tumor necrosis factor ligand superfamily member 10
Nec-1	Necrostatin-1
TNF-R1	Tumor necrosis factor receptor 1, tumor necrosis factor receptor superfamily member 1A
TNF-R2	Tumor necrosis factor receptor 2, tumor necrosis factor receptor superfamily member 1B
TRAIL-R1	Tumor necrosis factor-related apoptosis-inducing ligand receptor 1, tumor necrosis factor receptor superfamily member 10A
TRAIL-R2	Tumor necrosis factor-related apoptosis-inducing ligand receptor 2, tumor necrosis factor receptor superfamily member 10B
TRADD	TNFα receptor-associated death domain protein, tumor necrosis factor receptor type 1-associated death domain protein
TRAF2	TNFα receptor-associated factor 2, TNF receptor-associated factor 2
TRAF5	TNFα receptor-associated factor 5, TNF receptor-associated factor 5
IAP-1	Inhibitor of apoptosis protein 1, baculoviral IAP repeat-containing protein 3
IAP-2	Inhibitor of apoptosis protein 2, baculoviral IAP repeat-containing protein 2
NEMO	NF-kappa-B essential modulator
IKK	IκB kinase

Z. Dunai (✉) · R. Mihalik
Department of Pathogenetics, National Institute of Oncology,
Ráth György street 7-9,
Budapest H-1122, Hungary
e-mail: dunaizs@oncol.hu

P. I. Bauer
Department of Medical Biochemistry, Semmelweis University,
Tuzolto street 37-47,
Budapest H-1094, Hungary

TAK1	Transforming growth factor β-activated kinase 1
TAB2	TAK1 binding protein 2
NFkB	Nuclear factor NF-kappa-B
FADD	Fas-associated death domain protein
RIPK3	(also known RIP3) Receptor-interacting protein kinase 3, receptor-interacting serine/threonine-protein kinase 3
DISC	Death-inducing signaling complex
CYLD	Ubiquitin carboxyl-terminal hydrolase
RHIM	RIP homotypic interaction motif
PYGL	Glycogen phosphorylase
GLUL	Glutamine synthetase
GLUD1	Glutamate dehydrogenase 1
ROS	Reactive oxygen species
NOX-1	NADPH oxidase 1
BHA	Butylated hydroxyanisole
ANT	Adenine nucleotide translocator, ADP/ATP translocase
CYPD	Cyclophilin D
mPTP	Mitochondrial permeability transition pore
ATP	Adenosine triphosphate
APAF1	Apoptotic protease-activating factor 1
BAK	Bcl-2 homologous antagonist/killer
BAX	Bcl-2-like protein 4
DIF-1	Differentiation-inducing factor
atg1	Autophagy related 1 homolog gene
zVAD. fmk	Carbobenzoxy-valyl-alanyl-aspartyl-[O-methyl]- fluoromethylketone
NK cell	Natural killer cell
CCI	Controlled cortical impact
TBI	Traumatic brain injury
NMDA	N-Methyl-D-aspartate
LDH	Lactate dehydrogenase
GSH	Glutathione
AIF	Apoptosis-inducing factor
PAR	poly(ADP-ribose)chain
AA	Arachidonic acid
LOX	Lypoxigenase
JNK	c-Jun N-terminal kinase 1, mitogen-activated protein kinase 8
NO	Nitric oxide
MnSOD	Superoxide dismutase [Mn]
ACAT	Acyl-CoA:cholesterol acyltransferase
CHOP	C/EBP homologous protein
cFLIP	Cellular FLICE-like inhibitory protein, CASP8 and FADD-like apoptosis regulator
MDR-ABC	Multi-drug resistant ATP binding cassette
AML	Acute myeloid leukemia
GM-CSF	Granulocyte-macrophage colony stimulating factor
CAD	Caspase-activated DNAse
ICAD	Inhibitor of CAD
DED	Death effector domain
cFLIP	Cellular FLICE-inhibitory protein
ROCK1	Rho-associated protein kinase 1
RAIDD	Receptor-interacting protein-associated ICH-1/CED-3 homologous protein with a DD

Introduction

In 1972 Kerr, Wyllie and Currie proposed a controlled cell elimination process which acts complementary but opposite to cell division, to keep tissue homeostasis. That was suggested to be an active and programmed process which can be initiated and inhibited by various physiological or pathological stimuli [1]. Since then apoptosis, as they termed, became a widely investigated cell physiological process. Later Horvitz et al. described the molecular genetic pathway responsible for apoptosis that leads to genetically determined cell elimination during the development of the model organism *Caenorhabditis elegans*. Apoptosis has become a widely used term and is often considered to be synonymous with programmed cell death (PCD), while necrosis remained a cell death type lacking the morphological signs of apoptosis [2].

Conventional knowledge registers apoptosis to be a caspase-dependent, programmed, non-immunogenic process, characterized by cellular shrinkage, membrane blebbing, chromatin condensation and DNA degradation. On the contrary, necrosis is a deranged or accidental cell demise that involves increase in cell volume, swelling of organelles and early ruptures of plasma membrane. Autophagy is characterized by lack of chromatin condensation, redistribution of LC3 into autophagosomes membrane and accumulation of double-membrane covered vacuoles containing cytoplasmic organelles or cytosol [3].

As the experimental scope widened various sub-types of basic cell death forms were defined based not only on morphological criteria but considering other biochemical, functional or immunological aspects too. This resulted in new expressions such as caspase-independent cell death (CICD) [4], oncosis [5], necrapoptosis [6], necrotic-like cell death [7] or programmed necrosis [8] to define the variegated appearance of cell death types. Some of these terms refer to a cell death characterized by necrotic morphology but reported as a regulated event. Accumulating evidences imply that necrotic cell death can be a genetically regulated event and can be classified as programmed cell death in line with apoptosis. However: contrary to the fairly well characterized pathways of apoptosis the molecular composition of necrotic pathway (s) are hardly known.

A novel non-apoptotic caspase-independent cell demise form, termed necroptosis, has been described recently [9]. According to Degterev's model, necroptosis can be triggered in different cell lines by death ligand binding to its adequate receptor under caspase-compromised conditions. On the other hand necroptosis is characterized by morphology resembling to unregulated necrosis: early loss of plasma membrane integrity, gain in cell volume and swelling organelles. This model was based on earlier observations, for instance Vercammen and others reported that FasL (CD95L) treatment rendered the Fas receptor (CD95 receptor) transfected L929 murine cells more sensitive to necrosis when caspase activation was prevented [10]. Likewise FasL killed the activated primary T cells efficiently in the absence of active caspases, which results in necrotic morphological changes [11]. Caspase-8 deficient Jurkat cells underwent necrotic-like cell death after FasL exposure [12]. Moreover, TNFα treatment led to necrosis in L929 cells [13, 14]. It has also been shown previously that RIPK1 (RIP1) participated in the TNFα signaling cascades [15] and was also required for necrotic death induced by TNFα or TRAIL (Apo-2L) [11]. Degterev et al. developed a novel family of RIP kinase inhibitors namely necrostatins [9]. One of these, Nec-1 (Necrostatin-1) efficiently blocked necroptosis acting on RIPK1 via binding and freezing its inactive conformation. On the other hand Nec-1 did not inhibit apoptosis and thus provided an easy experimental way to distinguish between apoptosis and necroptosis [16]. Nec-1 was being used to investigate the pathological importance of this cell death type; pretreatment with Nec-1 reduced the unwanted cell loss in ischemic mice model [9]. This result revealed that necroptosis could contribute to the development of various diseases marked by necrotic phenotype like neurodegenerative disorders [17], viral infection [7], vascular occlusive neuronal diseases [18], myocardial infarction [19] and traumatic cell loss [20].

In the last few years various studies focused on the molecular background of necroptosis, but the role of necroptosis in diseases or physiological processes have just emerged. In this review we summarize the biochemical, physiological and pathological aspects of necroptosis and its possible function as an alternative way of antiviral defense mechanism in virus infected cells.

Biochemical Aspects

Death receptors, members of the tumor necrosis factor receptor superfamily, upon binding their respective ligands trigger pro-survival signals or apoptosis. Under special conditions TNF-R1, TNF-R2, Fas- and Apo-2L receptors TRAIL-R1 and TRAIL-R2 can initiate necrotic-like cell death [10, 21, 22]. The most extensively studied pathway leading to necroptosis is triggered by binding of TNFα to TNF-R1 (see review [23]). TNFα generally induces prosurvival signal through NFkB but is also able to induce apoptosis or necrotic-like cell death. Once TNFα is engaged by TNF-R1, the receptor trimerises and undergoes a rapid conformational change that provides anchoring site to TRADD and RIPK1 together with several different ubiquitin ligases such as TRAF2, TRAF5, IAP-1 or IAP-2 to form the so-called membrane-associated complex I [24]. In membrane-bound complex I E3 ligases catalyse the addition of Lys63-linked polyubiquitin moieties to Lys377 of RIPK1, creating a docking site for the TAK1–TAB2 complex that provides an attractive surface for recruitment of NEMO, a regulatory subunit of the IKK complex [25]. This leads to the activation of IKKα and IKKβ catalytic subunit further leading to the phosphorylation of IkB. Phosphorylated IkB is target for polyubiquitilation and subsequent proteasomal degradation thereby contributes to the release of NFkB that translocates to the nucleus and mediates prosurvival gene expressions and also the activation of MAPK cascade [26, 27]. Then the membrane-bound complex I internalizes while TRADD and RIP1 kinase dissociate from cytoplasmic supramolecular complex I and provide a docking surface for FADD, caspase-8 and RIPK3 hereby forming the cytoplasmic supramolecular complex II [28]. In complex II, caspase-8 cleaves RIP1 and RIP3 kinases and contributes to the fulfillment of apoptosis [29]. In parallel, a negative feedback loop of NFkB is proven by A20 a dual E3 ligase and ubiquitin hydrolase that cleaves off the K63-linked polyubiquitin chain from RIPK1 and subsequently marks RIPK1 for proteasomal degradation. This contributes to limit the proinflammatory activation of NFkB [23].

In the TRAIL- and FasL-induced signaling pathways, compared to the TNFα-induced one, different proteins participate in the formation of complex I but the composition of complex II seems to be similar [30]. While FADD was required for programmed necrosis induced by FasL or TRAIL in primary T cells [11] it was dispensable in case of TNFα-induced programmed necrosis [22]. This finding is coincidental with the distinct molecular composition of DISC or complex I during TRAIL, FasL or TNFα-induced apoptosis [24, 30–32].

Once FADD is recruited, it provides an attractive surface to bind the initiator procaspase-8/procaspase-10 and/or cFLIP (cellular FLICE-inhibitory protein) through the interactions of their homotypic death effector domains (DED). This contributes to the autoproteolytic activation of procaspase-8 and -10 to form active caspases. Active caspase-8 and -10 are released to the cytosol and they further activate the effector capsases, caspase-3, -6 and -7 which execute the apoptotic program performing the cellular and biochemical tasks leading to cell demise [33,

34]. For example, the internucleosomal cleavage of the DNA is performed by caspase-activated DNAse (CAD) through the cleavage of its inhibitor ICAD by effector caspases [35]. There are more than 400 proteins that are substrates of caspases among others ROCK1 which induces membrane blebbing or lamins which contribute to the chromatin condensation [36, 37]. cFLIP due its structural homology with procaspase-8 and procaspase-10 competitively prevents the recruitment of the initiator procaspases and therefore is able to arrest the downwards development of death receptor signals [38]. High level of cFLIP has been observed in different types of cancers [39, 40].

Under caspase-compromised conditions, at least in case of certain cell types (L929, U937, BALBc 3T3, MEF or Jurkat) TNFα exposure induces RIP kinase-dependent necroptosis, which can be block by Nec-1 a special inhibitor of RIP1 kinase activity [16, 21]. Hitomi et al. in an impressive genome-wide siRNA based study identified 432 genes involved in this process while seven of the isolated genes participate in apoptosis and necroptosis too, 32 genes are supposed to act downstream as regulator of RIPK1 [41]. One of those genes which expression is increased CYLD, a lysine 63 (K63) deubiquitinase which selectively removes polyubiquitin from RIPK1 is enhanced in necroptosis sensitive cells. The deubiquitylation of RIP1 kinase by CLYD promotes the formation of complex II and so helps the association of RIP1 and RIP3 kinases through their RIP homotypic interaction motifs (RHIM). It has been also shown that the activity of RIP3 kinase and the phosphorylation-driven assembly of RIP1 and RIP3 kinase complex is also required for necroptosis but is dispensable for apoptosis [28]. In concordance with this, sensitivity of cell lines for necroptosis correlates well with their RIP3 kinase expression [42]. The exact role of RIP3 kinase in complex II (also-called necrosome or pronecrotic complex II) remains unclear. It has been reported that RIP3 kinase also interacts with key enzymes of metabolic pathways including glycogen phosphorylase (PYGL), glutamine synthetase (GLUL), and glutamate dehydrogenase 1 (GLUD1) and increases their activity, leading to enhanced mitochondrial metabolism and indirectly elevated ROS production [43]. However, the origin and role of reactive oxygen species in necroptosis is still controversial. Some recent reports underscore the impact of plasma membrane-associated NADPH oxidase 1 (NOX-1) which contributes to ROS generation upon TNFα exposure [44]. In murine L929 cells knockdown of NOX-1 or treatment with antioxidant BHA (butylated hydroxyanisole) reduced the TNFα-induced necrosis [45], while in other cell types e.g. in U937 or Jurkat cells ROS scavenging failed to prevent necroptosis [9].

As another connection to cell metabolism it was revealed that RIP1 kinase can inhibit the activity of the mitochondrial ADP/ATP translocase (ANT) and therefore contributes to the depletion of cytoplasmic ATP level during necroptosis [46]. Since cyclophilin D (CYPD) interacts with ANT to form the mitochondrial permeability transition pore (mPTP), RIP1 kinase may alter the formation of mPTP.

The commitment and the executional phases of necroptosis are poorly explored. It was observed that the organelle swelling and plasma membrane rupture are similar to that of classical type III necrosis. The kinetics of changes in mitochondrial, lysosomal and plasma membrane integrity during distinct types of necrosis namely necroptosis, secondary necrosis and H_2O_2-induced necrosis were investigated by Vandenabeele et al. [47]. It was reported in an elegant comparative study that necroptosis and secondary necrosis have a longer signaling- and a shorter cellular disintegration phase. During the signaling phase gradually elevated ROS production, mitochondrial hyperpolarization was observed, which ended in a lysosomal membrane permeabilization and oxidative burst until the eventual loss of plasma membrane integrity. Contrarily H_2O_2-induced necrosis started with the disintegration phase, rapid ROS production, mitochondrial hyperpolarization and lysosomal membrane permeabilization. The authors concluded that all the three types of necrosis are characterized by the same subcellular events but with different kinetics [47].

To summarize up our knowledge, it seems that death receptor-initiated apoptotic and necrotic cell death machinery use a shared network of proteins. The selection of the participating molecules depends on the cell death inducing conditions and on cell types too, and a combination of those together with up till unknown factors will finally define the form of cell death process followed. This notion is confirmed by the common genes scored positively in siRNA screen studied to explore the participant genes in both apoptotic and necroptotic pathway [41]. Nevertheless, several questions remain unclear. Among others, late events leading to membrane rupture and the involvement of autophagy in the process of necroptosis are barely known [9, 48, 49].

Physiological Aspects

Apoptosis was described as a regulated cell death form which participates in normal tissue homeostasis during the embryogenesis of different organisms, especially in the genetic control of cell elimination during development of *C. elegans*. This early description of apoptosis may had influenced researchers to focus on the programmed cell death and at the same time neglect the other modalities of cell death already known [1, 2].

At the same time some arguments suggested that apoptosis is not the solely form of cell death responsible

for unwanted cell elimination during embryogenesis or physiological tissue renewal. For example interdigital membrane elimination provides a representative evidence for the role of apoptosis in tissue sculpting during embryonic development. Contrarily to this Yoshida et al. reported that a delayed but apparently normal interdigital membrane removal exists in APAF1$^{-/-}$ mutant mice embryos, where the apoptosome cannot be formed [50].

In other cases, necrotic-like cell demise was observed under conditions where the apoptotic machinery was blocked. For instance, in BAK$^{-/-}$ and BAX$^{-/-}$ double mutant mice various defects of apoptotic process were observed: the syndactyly phenotype, excess cells accumulation in the central nervous system and finally most embryos died at or just before birth. Some of the littermates of the dying BAK$^{-/-}$ and BAX$^{-/-}$ mice could survive and lived until adulthood [51, 52]. Similarly, the protist *Dictyostelium discoideum* stalk cells showed autophagy-like cell death when they were treated with differentiation-inducing factor (DIF-1) under condition of starvation. Inactivation of autophagy-related 1 homolog gene (*atg1*) shifted the process to a classical necrotic-like cell death [53]. Based on this results necrosis is a candidate process as a back-up mechanism which can substitute the defective apoptotic pathway. On the other hand, necrosis was also observed during normal mammalian tissue homeostasis as a programmed event. For instance, the human large intestine crypts contain necrotic colonocytes [54], or in human growth plate during late pubertal fusing, chondrocytes showed necrotic phenotype [55]. Moreover, disappearance of linker cells in *C. elegans* at a well defined time point during development is essential for future fertility. Abraham et al. reported that caspases and other apoptosis-associated gene products did not participate in the loss of linker cells and indeed, the dying linker cells showed necrotic morphology [56, 57]. For more details see reviews of Kroemer, McCall and Thompson [58–60]. However, the above-mentioned reports are based mostly on morphological observations, therefore further investigations would be preferable, to evaluate the type of cell death.

This context raises the question of evolutional appearance and potential hierarchy of different cell death modalities. Golstein and Kroemer discussed the redundancy of cell death mechanisms and hypothesized that an evolutionary ancestral cell death—resembling necrosis—was overridden by evolutionarily younger, more elaborate processes, likely autophagy and apoptosis. Cell death types emerging later in evolution may turn into the major dominant form of cell death while masking the ancestral one, but which can resurface as back-up mechanism when the other cell death pathways are blocked [61].

It is tempting to speculate how necroptosis is related to other cell death modalities and what physiological role it might has. It is conceivable that necroptosis compared to necrosis is a relatively new evolutionary mechanism, an alternative programmed cell death that serves as a back-up process when apoptosis is abrogated. Hitomi et al. in a murine genome-wide siRNA screen for genes involved in zVAD.fmk-induced necroptosis in L929 cell line have found that some genes required for necroptosis had increased expression in macrophages, dendritic cells and NK cells, while another gene cluster was upregulated in B and T lymphocytes connecting necroptosis to the immunological processes [41]. Another interaction between the necroptotic process and the immune-response was reported [28]. Viruses are known to frequently express antiapoptotic proteins to control the lifespan of their (Vertebrate) host in order to avoid cell death until their replication cycle is finished [62]. As Cho et al. showed that in vaccinia virus infected T cell, when the apoptotic process was abrogated by caspase inhibitors, the RIP3K-directed necroptotic cell death became dominant [28]. Accordingly, necroptosis might be an evolutionary response of host cells to avoid the pathogen-induced apoptosis inhibition. Moreover, further benefit of the necrotic cell demise in a multicellular organism might be that necrosis/necroptosis leads to a proinflammatory response. Release of the intracellular content can provoke the immune response, which serves not only as a warning system, indicating the internal or external hazardous conditions, but also initiates the immune response. This observation raises the clinical benefits of necroptosis induction in antiviral therapy.

To study the phylogenetics of necroptosis, the Inparanoid, a comprehensive database of eukaryotic orthologs was used and results showed that RIP1 and RIP3 kinases are characteristic to Vertebrates. However, a human RIP1 kinase homolog can be found in an annelid worm (*Capitella sp.*), and proteins similar to the human RIP3 kinase is present in the chordate *Ciona savignyi*, the amoeba *Dictyostelium discoideum*, and in the smallest free-living unicellular eukaryote, *Ostreococcus tauri* [63]. The connection of those proteins to cell death pathways are unknown and their homology might be explained by prior exon shuffling process. Based on the above mentioned facts necroptosis may be an important stage in the arms race where vertebrates are constantly participating, evolving and learning how to cope with viral invasion.

The above mentioned evidences namely that certain cell types can initiate their own death via necrosis, apparently refute the bias that the term necrosis solely refers to accidental, uncontrolled event.

Pathological Aspects

Necrosis has for a very long time been described as a direct cause or as a simultaneously occurring phenomenon of cell death in many cases of human diseases, such as neurodegenerative disease [17], pancreatitis [64], trauma [20],

ischemia reperfusion in heart attack [19] or in brain injury [65]. However, having the preconception that this type of cell death is random and unregulated, researchers have regarded its harmful effects inevitable. Undoubtedly, overwhelming stress induces necrosis that cannot be impeded, it is still worthwhile to examine if there is an activated signaling pathway behind the focal necrotic morphology that at least partially can be hampered. For instance following ischemia in the brain [66] and myocardial tissues [67] an increase in inflammatory cytokines production, including TNFα might occur and resulting in accompanying necroptosis.

Using Necrostatin-1 Degterev et al. investigated the in vivo participation of necroptosis in a murine model of ischemia reperfusion [9]. As it was reported the area of the infarct and the neurological score due to middle cerebral artery occlusion was significantly reduced by intracerebroventricular administration of 7-Cl-Nec-1 in a wide therapeutic window [9]. On the other hand, Whalen et al. hypothesized that Necrostatin-1 would reduce histopathological appearance of cell death and improve functional outcome in a controlled cortical impact (CCI) model of traumatic brain injury (TBI) in mice. As they reported, short- and long-term Nec-1 treatment attenuated the plasma membrane damage and moderated the motor and cognitive deficits compared to untreated littermates after CCI. In similar experiments Nec-1 failed to adjust the outcomes the caspase-mediated apoptosis which also operates in TBI [20]. Moreover, pretreatment with Nec-1 significantly reduced the microglial activation and neutrophil influx 48 h after CCI. These results underline the possible involvement of necroptosis in acute brain injury and underscore the applicability of Nec-1 in reducing posttraumatic lesion and inflammation [68].

Additional contribution of necroptosis in neurodegenerative diseases has been published recently. NMDA exposure through ionotropic glutamate receptor-induced excitotoxicity in primary rat cortical cell cultures could be partially reduced by Nec-1, shown by cell viability assays and LDH leakage. It was also reported by the authors that the elevated intracellular Ca^{++} level may contribute to NMDA-induced necroptosis [69].

Oxidative glutamate toxicity, also called as oxytosis is characterized by GSH depletion, increased reactive oxygen species production, calcium influx and caspase-independence that could be prevented by antioxidants [70]. Further study showed that Nec-1 could attenuate non-receptor-mediated glutamate toxicity in a mouse hippocampal cell line (HT22) and not only blocked the nuclear translocation of apoptosis-inducing factor (AIF) but an increase in the GSH level and a decrease of ROS production were also observed [71]. The inhibition of AIF transport to the nucleus by Nec-1 raises the question whether is there a connection between RIP1 kinase activity and AIF transport, thus PAR plays any role in the necroptotic process [72]. These questions need further experimental answers.

In another study Kim et al. characterized arachidonic acid-(AA) induced cell death in oligodendrocyte precursors [73]. The authors previously demonstrated that AA-induced cell death was lypoxigenase- (LOX) dependent and mediated by elevated ROS level and JNK activation, but it was independent from caspase activation. Authors confirmed that arachidonic acid-induced cytotoxicity was markedly prevented by an antioxidant BHA, or by a 12-LOX specific inhibitor or by Nec-1. Moreover, Nec-1 markedly prevented cell death via blocking ROS production and JNK activation. The authors hypothesized that deubiquitylation of RIP1 kinase is upregulated by oxidative stress contributing to necroptosis. This is supported by the notion that Nec-1 also blocked oxidative cell death that was induced by glutathione-depleting agents. This and previously mentioned findings underline the possibility of the receptor-independent role of RIP1 kinase in oxidative stress.

Peroxynitrite formed from NO and superoxide are among the most damaging nitrosative agents. In rat pulmonary endothelial cells the superoxide was formed as a by-product of mitochondrial respiration in sufficient amount to generate peroxynitrite in the presence of NO. Peroxynitrite inhibited mitochondrial complex I via S-nitrosation of NDUFB8 (a protein presents in complex I), which limited electron entry into the mitochondrial electron transport chain and led to endothelial dysfunction. NO-induced necrotic cell death could be prevented by overexpression of MnSOD or inhibition of RIP1 kinase by Nec-1. The level of RIP3 kinase was elevated by NO treatment and was colocalized to mitochondria. These results further support the role of RIP1 and RIP3 kinase in receptor independent NO-induced necroptosis [74].

Smith and colleagues tested the protective effect of Nec-1 on animal cardiomyocite cell lines and in different in vivo murine models in case of heart and brain ischemia reperfusion injury [75]. They reported that Nec-1 could reduce the infarct size and was protective against ischemia reperfusion injury in the myocardium. In their subsequent paper they demonstrated that the CYPD, a core component of mPTP, had to be present in order to observe cardyoprotective effect by Nec-1 [76]. In wild-type mice Nec-1 efficiently reduced infarct size in vivo when administered intravenously during reperfusion but it failed to produce a similar effect in $CYPD^{-/-}$ mice due to its resistance for mPTP formation ability [76]. This corresponds to results discovering that RIPK1 may alter mPTP formation [46].

It was recently published that cell death induced by sitosterol, a plant sterol, differs from the cholesterol-induced cell death modality in isolated murine macrophages [77].

Cholesterol triggers a caspase-, acyl-CoA:cholesterol acyltransferase- (ACAT), C/EBP homologous protein- (CHOP) and JNK-dependent apoptosis via endoplasmatic reticulum stress. In contrast, sitosterol, which differs from cholesterol in a single ethyl group, initiates a caspase-independent, p38-dependent process which could be completely blocked by Nec-1 and moderately by autophagy inhibitors. The authors hypothesized that in sitosterolemia the accumulating sitosterol may lead to accelerated macrophage death resembling necroptosis, coupled with inefficient phagocytic clearance. This contributes to a faster progression of plaque necrosis, disruption and thrombo-occlusive vascular events which are common symptoms in sitosterolemia [77].

A potential anticancer drug shikonin, a naphtoquinone isolated from *Lithospermum erythrorhizon* was found to induce necroptosis in MCF-7 adenocarcinoma and HEK293 embryonic kidney cells. Nec-1 effectively inhibited the shikonin-induced loss of plasma membrane integrity, mitochondrial depolarization, elevation of ROS level, and formation of autophagyc vacuoles. Interestingly, the shikonin-triggered necroptosis showed faster kinetics compared to the TNFα-induced one reported by Degterev et al. This phenomenon can be explained by direct activation of necroptosis in case of shikonin, compared to the effect of TNFα which induces first apoptotic cell death and which later turns into necroptosis in presence of caspase inhibitor [78].

Induction of cell death is a double edged sword. While in the case of neurological disorders prevention of cell death is in the focus of therapeutically approaches, in case of cancer cells the elimination of excess cell growth is in the frontline. Deregulated apoptosis is a hallmark of cancer [79]. A decrease in the sensitivity to apoptosis induction is an important stage of carcinogenesis, elevated level of antiapoptotic proteins (BCL-2, BCL-Xl, cFLIP, IAPs, heat shock proteins) or reduced expression or mutations of proapoptotic proteins (P53, APAF1, BAX, BAD, caspases, death ligands, death receptors) provide the means for that [79]. Therefore it became an attractive option in theory to trigger different cell death pathways to kill tumor cells. Several apoptosis inducer are successfully applied in the anticancer therapy [80, 81]. However acquired drug resistance due to high ABC transporter expression in tumors is the reason of poor outcome in clinical practice. Therefore finding necroptosis-inducing drugs which are not targets of MDR-ABC transporters would have high clinical relevance.

Necroptosis as being a back-up mechanism of apoptosis may represent a new perspective in anticancer therapy. Recently published data revealed that in glucocorticoid resistant acute lymphoblastic leukemia (ALL) cells can be resensitized by obatolax—antagonist of Bcl-2 family—via autophagy-dependent necroptotic cell death [49]. Thus, activation of autophagy is required to induce necroptosis. Involvement of RIP1 kinase, CYLD and autophagy-related proteins indicate a direct link between the necroptotic and autophagyc network [49].

It was shown that in AML a GM-CSF-fused diphtheria toxin construct initiated simultaneously apoptosis and necroptosis [82]. Cell death induced by diphtheria toxin alone in three AML cell lines were similar to the cycloheximide-triggered apoptosis which was moderately inhibited by zVAD.fmk or Nec-1. These results indicate the role of RIP1 kinase in diphteria toxin-induced cell death and may signify the contribution of a freshly synthesized protein in necroptosis.

Concluding Remarks

A few years ago necrotic cell death was considered as a consequence of cell injuries that resulted in an irreversible energetic failure of the cell. The term necrosis is often associated to pathological cell loss and therefore believed to be harmful. Accumulating evidences indicate that necrotic cell death can be a regulated event too. As we discussed above, necroptosis, as an alternative form of programmed necrosis might be the dominant form of cell death under physiological and pathological conditions activated by various stimuli. Growing body of evidences provide a more and more detailed picture from the underlying molecular mechanisms of the cell death process, however up till now, many questions remain unresolved.

Besides the theory that necroptosis can serve as a back-up mechanism which can supplement apoptosis, direct initiation of necroptosis was also shown. This pathway might have faster kinetics than the indirectly activated process. However, there are only few information available considering necroptosis as a primary pathway due to the prevalent view which defines necroptosis as a cell death ligand-induced process, which occurs only under caspase-compromised conditions. More experimental results are needed for changing of this view.

The central role of RIP1 kinase in necroptotic process is firmly established. Most of the publications deal with the death ligand-induced activation of RIP1 kinase, but recent evidences underlines that there might be a receptor-independent activation pathway of RIP kinases too. According to the known pathway, after TNFα exposure RIP1 kinase is recruited to the cytosolic complex II after its deubiqutylation directly from the membrane-associated complex I together with TRADD. Hypothetically RIPK1 might be alternatively activated in a complex I-independent manner too. In this case the origin of the surface serving as

initial site of complex II formation is an enigma but recent evidences predict its existence [72–74].

Such cytosolic complex is the PIDDosome which is composed of the p53-inducible protein (PIDD), caspase-2 and the adaptor protein RAIDD and is thought to play a role in p53-, or DNA damage-mediated apoptosis [83]. A recent study indicated a possible role of PIDD in the NFkB-mediated survival [84]. Upon genotoxic stress, RIPK1 and NEMO were also observed to be recruited to the PIDDosome, suggesting that PIDD acts as a molecular switch between apoptosis and survival. However Ohashi et al. reported that PIDD is dispensable for DNA damage-induced apoptosis and for NFkB-mediated survival pathway too [85]. Since RIP1 kinase plays critical role in necroptosis it is tempting to speculate whether RIP1 kinase acts through its kinase activity in the PIDDosome and also about the possible role of the PIDDosome in the necroptotic pathway.

Further question is the demarcation of different types of programmed necrosis. The term programmed necrosis has also been used to describe poly (ADP-ribose) polymerase-1 (PARP1)-mediated cell death induced by DNA damaging agents [86, 87].

Moreover, the involvement and contribution of autophagy to necroptosis is also controversial. While Degterev et al. reported that autophagy is a common downstream consequence of necroptosis and acts as a scavenger of cellular debris, other report has shown that inhibition of autophagy blocks the necroptotic process too [9, 49]. This paradoxon might be explained by considering that in different cell lines the two cell death process might be subsequent, in other cases parallel events. This might depends on the different sets of available proteins in different cell types.

One of the well known characteristics of cancer cells is their ability to avoid apoptosis [79]. Scientists recently have begun to consider whether activating necrosis might be a beneficial outcome in anticancer therapy. Partly through the killing of tumor cells and also by boosting the immune system's activity through the spilled out content of dying cells. On the other hand in a recent update review of Hanahan and Weinberg expressed their view that the necrotic process might have disadvantages, namely the activation of immune system due to the spill out of intracellular content of dying cell might have promote formation of neoplasia as it has been observed in case of chronic inflammation, instead of killing the existing tumor cells [88, 89].

Both predictions need further experimental proofs, a better knowledge of the switching mechanisms between apoptosis and necroptosis. Than the decision to choose between different forms of cell death might be the part of personalized treatment in the fight against cancer.

Acknowledgements This work was supported by grant ETT 016/2008 for PIB.

References

1. Kerr JF, Wyllie AH, Currie AR (1972) Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br J Cancer 26(4):239–257
2. Metzstein MM, Stanfield GM, Horvitz HR (1998) Genetics of programmed cell death in C. elegans: past, present and future. Trends Genet 14(10):410–416
3. Kroemer G, El-Deiry WS, Golstein P, Peter ME, Vaux D, Vandenabeele P et al (2005) Classification of cell death: recommendations of the nomenclature committee on cell death. Cell Death Differ 12(suppl 2):1463–1467
4. Déas O, Dumont C, MacFarlane M, Rouleau M, Hebib C, Harper F et al (1998) Caspase-independent cell death induced by anti-CD2 or staurosporine in activated human peripheral T lymphocytes. J Immunol 161(7):3375–3383
5. Majno G, Joris I (1995) Apoptosis, oncosis, and necrosis: an overview of cell death. Am J Pathol 146(1):3–15
6. Lemasters JJ (1999) V. Necrapoptosis and the mitochondrial permeability transition: shared pathways to necrosis and apoptosis. Am J Physiol 276(1):G1–6
7. Li M, Beg AA (2000) Induction of necrotic-like cell death by tumor necrosis factor alpha and caspase inhibitors: novel mechanism for killing virus-infected cells. J Virol 74(16):7470–7477
8. Borst P, Rottenberg S (2004) Cancer cell death by programmed necrosis? Drug Resist Updat 7(6):321–324
9. Degterev A, Huang Z, Boyce M, Li Y, Jagtap P, Mizushima N et al (2005) Chemical inhibitor of nonapoptotic cell death with therapeutic potential for ischemic brain injury. Nat Chem Biol 1 (2):112–119
10. Vercammen D, Brouckaert G, Denecker G, Van De Craen M, Declercq W, Fiers W et al (1998) Dual signaling of the Fas receptor: initiation of both apoptotic and necrotic cell death pathways. J Exp Med 188(5):919–930
11. Holler N, Zaru R, Micheau O, Thome M, Attinger A, Valitutti S et al (2000) Fas triggers an alternative, caspase-8-independent cell death pathway using the kinase RIP as effector molecule. Nat Immunol 1(6):489–495
12. Kawahara A, Ohsawa Y, Matsumura H, Uchiyama Y, Nagata S (1998) Caspase-independent cell killing by Fas-associated protein with death domain. J Cell Biol 143(5):1353–1360
13. Laster SM, Wood JG, Gooding LR (1988) Tumor necrosis factor can induce both apoptic and necrotic forms of cell lysis. J Immunol 141(8):2629–2634
14. Schulze-Osthoff K, Krammer PH, Dröge W (1994) Divergent signalling via APO-1/Fas and the TNF receptor, two homologous molecules involved in physiological cell death. EMBO J 13 (19):4587–4596
15. Hsu H, Huang J, Shu HB, Baichwal V, Goeddel DV (1996) TNF-dependent recruitment of the protein kinase RIP to the TNF receptor-1 signaling complex. Immunity 4(4):387–396
16. Degterev A, Hitomi J, Germscheid M, Ch'en IL, Korkina O, Teng X et al (2008) Identification of RIP1 kinase as a specific cellular target of necrostatins. Nat Chem Biol 4(5):313–321
17. Yuan J (2009) Neuroprotective strategies targeting apoptotic and necrotic cell death for stroke. Apoptosis 14(4):469–477
18. Neumar RW (2000) Molecular mechanisms of ischemic neuronal injury. Ann Emerg Med 36(5):483–506
19. McCully JD, Wakiyama H, Hsieh YJ, Jones M, Levitsky S (2004) Differential contribution of necrosis and apoptosis in myocardial ischemia-reperfusion injury. Am J Physiol Heart Circ Physiol 286 (5 55–5):H1923–H1935
20. Zhang X, Chen Y, Jenkins LW, Kochanek PM, Clark RSB (2005) Bench-to-bedside review: apoptosis/programmed cell death triggered by traumatic brain injury. Crit Care 9(1):66–75

21. Vercammen D, Beyaert R, Denecker G, Goossens V, Van Loo G, Declercq W et al (1998) Inhibition of caspases increases the sensitivity of L929 cells to necrosis mediated by tumor necrosis factor. J Exp Med 187(9):1477–1485
22. Chan FKM, Shisler J, Bixby JG, Felices M, Zheng L, Appel M et al (2003) A role for tumor necrosis factor receptor-2 and receptor-interacting protein in programmed necrosis and antiviral responses. J Biol Chem 278(51):51613–51621
23. Andera L (2009) Signaling activated by the death receptors of the TNFR family. Biomed Pap 153(3):173–180
24. Micheau O, Tschopp J (2003) Induction of TNF receptor I-mediated apoptosis via two sequential signaling complexes. Cell 114(2):181–190
25. Ea CK, Deng L, Xia ZP, Pineda G, Chen ZJ (2006) Activation of IKK by TNFα requires site-specific ubiquitination of RIP1 and polyubiquitin binding by NEMO. Mol Cell 22(2):245–257
26. Chen ZJ (2005) Ubiquitin signalling in the NF-kB pathway. Nat Cell Biol 7(8):758–765
27. Vandenabeele P, Galluzzi L, Vanden Berghe T, Kroemer G. (2010) Molecular mechanisms of necroptosis: an ordered cellular explosion. Nat Rev Mol Cell Biol 11(10):700–714
28. Cho Y, Challa S, Moquin D, Genga R, Ray TD, Guildford M et al (2009) Phosphorylation-driven assembly of the RIP1-RIP3 complex regulates programmed necrosis and virus-induced inflammation. Cell 137(6):1112–1123
29. Feng S, Yang Y, Mei Y, Ma L, Zhu DE, Hoti N et al (2007) Cleavage of RIP3 inactivates its caspase-independent apoptosis pathway by removal of kinase domain. Cell Signal 19(10):2056–2067
30. Jin Z, El-Deiry WS (2006) Distinct signaling pathways in TRAIL- versus tumor necrosis factor-induced apoptosis. Mol Cell Biol 26 (21):8136–8148
31. Harper N, Hughes M, MacFarlane M, Cohen GM (2003) Fas-associated death domain protein and caspase-8 are not recruited to the tumor necrosis factor receptor I signaling complex during tumor necrosis factor-induced apoptosis. J Biol Chem 278 (28):25534–25541
32. Morgan MJ, Kim YS, Liu ZG (2009) Membrane-bound Fas ligand requires RIP1 for efficient activation of caspase-8 within the death-inducing signaling complex. J Immunol 183(5):3278–3284
33. Lavrik IN. (2011) Regulation of death receptor-induced apoptosis induced via CD95/Fas and other death receptors. Mol Biol 45 (1):150–155
34. Gonzalvez F, Ashkenazi A. (2010) New insights into apoptosis signaling by Apo2L/TRAIL. Oncogene 29(34):4752–4765
35. Sakahira H, Enari M, Nagata S (1998) Cleavage of CAD inhibitor in CAD activation and DNA degradation during apoptosis. Nature 391(6662):96–99
36. Lüthi AU, Martin SJ (2007) The CASBAH: a searchable database of caspase substrates. Cell Death Diff 14(4):641–650
37. Bulat N, Widmann C (2009) Caspase substrates and neurodegenerative diseases. Brain Res Bull 80(4–5):251–267
38. Irmler M, Thome M, Hahne M, Schneider P, Hofmann K, Steiner V et al (1997) Inhibition of death receptor signals by cellular FLIP. Nature 388(6638):190–195
39. Oosten ASV, Navis G, Stegeman CA, Joles JA, Klok PA, Kuipers F et al (2001) Increased expression of cFLIPL in colonic adenocarcinoma. J Pathol 194(1):15–19
40. Tepper CG, Seldin MF (1999) Modulation of caspase-8 and FLICE-inhibitory protein expression as a potential mechanism of Epstein-Barr virus tumorigenesis in Burkitt's lymphoma. Blood 94 (5):1727–1737
41. Hitomi J, Christofferson DE, Ng A, Yao J, Degterev A, Xavier RJ et al (2008) Identification of a molecular signaling network that regulates a cellular necrotic cell death pathway. Cell 135(7):1311–1323
42. He S, Wang L, Miao L, Wang T, Du F, Zhao L et al (2009) Receptor interacting protein Kinase-3 determines cellular necrotic response to TNF-α. Cell 137(6):1100–1111
43. Zhang DW, Shao J, Lin J, Zhang N, Lu BJ, Lin SC et al (2009) RIP3, an energy metabolism regulator that switches TNF-induced cell death from apoptosis to necrosis. Science 325(5938):332–336
44. Vanden Berghe T, Declercq W, Vandenabeele P (2007) NADPH Oxidases: new players in TNF-induced necrotic cell death. Mol Cell 26(6):769–771
45. Kim YS, Morgan MJ, Choksi S, Liu ZG (2007) TNF-induced activation of the Nox1 NADPH oxidase and its role in the induction of necrotic cell death. Mol Cell 26(5):675–687
46. Temkin V, Huang Q, Liu H, Osada H, Pope RM (2006) Inhibition of ADP/ATP exchange in receptor-interacting protein-mediated necrosis. Mol Cell Biol 26(6):2215–2225
47. Berghe TV, Vanlangenakker N, Parthoens E, Deckers W, Devos M, Festjens N et al. (2010) Necroptosis, necrosis and secondary necrosis converge on similar cellular disintegration features. Cell Death Differ 17(6):922–930
48. Yu L, Alva A, Su H, Dutt P, Freundt E, Welsh S et al (2004) Regulation of an ATG7-beclin 1 program of autophaglic cell death by caspase-8. Science 304(5676):1500–1502
49. Bonapace L, Bornhauser BC, Schmitz M, Cario G, Ziegler U, Niggli FK et al. (2010) Induction of autophagy-dependent necroptosis is required for childhood acute lymphoblastic leukemia cells to overcome glucocorticoid resistance. J Clin Invest 120 (4):1310–1323
50. Yoshida H, Kong YY, Yoshida R, Elia AJ, Hakem A, Hakem R et al (1998) Apaf1 is required for mitochondrial pathways of apoptosis and brain development. Cell 94(6):739–750
51. Lindsten T, Ross AJ, King A, Zong WX, Rathmell JC, Shiels HA et al (2000) The combined functions of proapoptotic Bcl-2 family members Bak and Bax are essential for normal development of multiple tissues. Mol Cell 6(6):1389–1399
52. Lindsten T, Thompson CB (2006) Cell death in the absence of Bax and Bak. Cell Death Differ 13(8):1272–1276
53. Laporte C, Kosta A, Klein G, Aubry L, Lam D, Tresse E et al (2007) A necrotic cell death model in a protist. Cell Death Differ 14(2):266–274
54. Barkla DH, Gibson PR (1999) The fate of epithelial cells in the human large intestine. Pathology 31(3):230–238
55. Emons J, Chagin AS, Hultenby K, Zhivotovsky B, Wit JM, Karperien M et al (2009) Epiphyseal fusion in the human growth plate does not involve classical apoptosis. Pediatr Res 66(6):654–659
56. Kumar A, Rothman J (2007) Cell death: hook, line and linker. Curr Biol 17(8):R286–R289
57. Abraham MC, Lu Y, Shaham S (2007) A morphologically conserved nonapoptotic program promotes linker cell death in Caenorhabditis elegans. Dev Cell 12(1):73–86
58. Yuan J, Kroemer G. (2010) Alternative cell death mechanisms in development and beyond. Genes Dev 24(23):2592–2602
59. McCall K. (2010) Genetic control of necrosis-another type of programmed cell death. Curr Opin Cell Biol 22(6):882–888
60. Zong WX, Thompson CB (2006) Necrotic death as a cell fate. Genes Dev 20(1):1–15
61. Golstein P, Kroemer G (2005) Redundant cell death mechanisms as relics and backups. Cell Death Differ 12(suppl 2):1490–1496
62. Benedict CA, Norris PS, Ware CF (2002) To kill or be killed: viral evasion of apoptosis. Nat Immunol 3(11):1013–1018
63. Berglund AC, Sjolund E, Östlund G, Sonnhammer ELL (2008) InParanoid 6: eukaryotic ortholog clusters with inparalogs. Nucleic Acids Res 36(suppl 1):D263–D266
64. Mareninova OA, Sung KF, Hong P, Lugea A, Pandol SJ, Gukovsky I et al (2006) Cell death in pancreatitis: caspases protect from necrotizing pancreatitis. J Biol Chem 281(6):3370–3381

65. West T, Atzeva M, Holtzman DM (2006) Caspase-3 deficiency during development increases vulnerability to hypoxic-ischemic injury through caspase-3-independent pathways. Neurobiol Dis 22(3):523–537
66. Stoll G, Jander S, Schroeter M (2002) Detrimental and beneficial effects of injury-induced inflammation and cytokine expression in the nervous system. Adv Exp Med Biol 513:87–113
67. Zhang M, Chen L (2008) Status of cytokines in ischemia reperfusion induced heart injury. Cardiovasc Hematol Disord Drug Targets 8(3):161–172
68. You Z, Savitz SI, Yang J, Degterev A, Yuan J, Cuny GD et al (2008) Necrostatin-1 reduces histopathology and improves functional outcome after controlled cortical impact in mice. J Cereb Blood Flow Metab 28(9):1564–1573
69. Li Y, Yang X, Ma C, Qiao J, Zhang C (2008) Necroptosis contributes to the NMDA-induced excitotoxicity in rat's cultured cortical neurons. Neurosci Lett 447(2–3):120–123
70. Tan S, Schubert D, Maher P (2001) Oxytosis: a novel form of programmed cell death. Curr Top Med Chem 1(6):497–506
71. Xu X, Chua CC, Kong J, Kostrzewa RM, Kumaraguru U, Hamdy RC et al (2007) Necrostatin-1 protects against glutamate-induced glutathione depletion and caspase-independent cell death in HT-22 cells. J Neurochem 103(5):2004–2014
72. Cregan SP, Dawson VL, Slack RS (2004) Role of AIF in caspase-dependent and caspase-independent cell death. Oncogene 23(16 REV. ISS. 2):2785–2796
73. Kim S, Dayani L, Rosenberg PA, Li J. (2010) RIP1 kinase mediates arachidonic acid-induced oxidative death of oligodendrocyte precursors. Int J Physiol Pathophysiol Pharmacol 2(2):137–147
74. Davis CW, Hawkins BJ, Ramasamy S, Irrinki KM, Cameron BA, Islam K et al. (2010) Nitration of the mitochondrial complex I subunit NDUFB8 elicits RIP1- and RIP3-mediated necrosis. Free Radic Biol Med 48(2):306–317
75. Smith CCT, Davidson SM, Lim SY, Simpkin JC, Hothersall JS, Yellon DM (2007) Necrostatin: a potentially novel cardioprotective agent? Cardiovasc Drugs Ther 21(4):227–233
76. Lim SY, Davidson SM, Mocanu MM, Yellon DM, Smith CCT (2007) The cardioprotective effect of necrostatin requires the cyclophilin-D component of the mitochondrial permeability transition pore. Cardiovasc Drugs Ther 21(6):467–469
77. Bao L, Li Y, Deng SX, Landry D, Tabas I (2006) Sitosterol-containing lipoproteins trigger free sterol-induced caspase-independent death in ACAT-competent macrophages. J Biol Chem 281(44):33635–33649
78. Han W, Li L, Qiu S, Lu Q, Pan Q, Gu Y et al (2007) Shikonin circumvents cancer drug resistance by induction of a necroptotic death. Mol Cancer Ther 6(5):1641–1649
79. Hanahan D, Weinberg RA (2000) The hallmarks of cancer. Cell 100(1):57–70
80. Hu W, Kavanagh JJ (2003) Anticancer therapy targeting the apoptotic pathway. Lancet Oncol 4(12):721–729
81. Li QX, Yu DH, Liu G, Ke N, McKelvy J, Wong-Staal F (2008) Selective anticancer strategies via intervention of the death pathways relevant to cell transformation. Cell Death Differ 15 (8):1197–1210
82. Horita H, Frankel AE, Thorburn A (2008) Acute myeloid leukemia-targeted toxin activates both apoptotic and necroptotic death mechanisms. PLoS ONE 3(12):e3909
83. Tinel A, Tschopp J (2004) The PIDDosome, a protein complex implicated in activation of caspase-2 in response to genotoxic stress. Science 304(5672):843–846
84. Janssens S, Tinel A, Lippens S, Tschopp J (2005) PIDD mediates NF-kB activation in response to DNA damage. Cell 123(6):1079–1092
85. Kim IR, Murakami K, Chen NJ, Saibil SD, Matysiak-Zablocki E, Elford AR et al (2009) DNA damage- and stress-induced apoptosis occurs independently of PIDD. Apoptosis 14(9):1039–1049
86. Tu HC, Ren D, Wang GX, Chen DY, Westergard TD, Kim H et al (2009) The p53-cathepsin axis cooperates with ROS to activate programmed necrotic death upon DNA damage. Proc Natl Acad Sci USA 106(4):1093–1098
87. Zong WX, Ditsworth D, Bauer DE, Wang ZQ, Thompson CB (2004) Alkylating DNA damage stimulates a regulated form of necrotic cell death. Genes Dev 18(11):1272–1282
88. Hanahan D, Weinberg RA. (2011) Hallmarks of cancer: the next generation. Cell 144(5):646–674
89. Grivennikov SI, Greten FR, Karin M. (2010) Immunity, inflammation, and cancer. Cell 140(6):883–899