



## **Cell Death and Its Different Modes: History of Understanding and Current Trends**

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*This work was carried out in collaboration between both authors. Author AVK designed the study, managed initial literature searches and revised the manuscript. Author AS analyzed and interpreted data, developed and wrote the manuscript. Both authors read and approved the final manuscript.*

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### **ABSTRACT**

Discussions about what is life continue to struggle; there are pros and cons for whether a virus is alive. However, an opposite thing – cell death – appears to be tantamount important and equally not-easygoing to define. Nevertheless, our current knowledge about eukaryotic cell death has made a long way and resulted in a fruitful outcome: starting from three types of cell death (type I, II and III which are mainly applicable to eukaryotic cells of organisms from the biological kingdom animalia) in 1970s, Nomenclature Committee on Cell Death has named already twelve cell death forms in 2018, including the above mentioned apoptosis, autophagy and necrosis among them. How the scientific attitude towards cellular demise evolved and various aspects of different cell death modes are reviewed in this article.

**Keywords:** *Nomenclature; regulated cell death; cornification; excitotoxicity; cysteine proteases; lysosome; plasma membrane; cancer.*

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## ABBREVIATIONS

*ACD* : Accidental cell death  
*ADCD* : Autophagy-dependent cell death  
*ATP* : Adenosine triphosphate  
*DAMP* : Damage-associated molecular pattern  
*NCCD* : Nomenclature Committee on Cell Death  
*RCD* : Regulated cell death  
*ROS* : Reactive oxygen species

## 1. INTRODUCTION

Today, our knowledge about eukaryotic cell death has a profound history. Microscopy of mammalian cell cultures, live tissues and stained sectioned specimens of various multicellular organisms (nematode *C. elegans*, fruit fly *D. melanogaster*, mouse, human and other) revealed many secrets of cellular life and death. Starting from three types of cell death (type I, II and III) in 1970's [1], cell death has been gaining interest at an increasing rate. Regulated cell death (RCD) or the events that resemble it have been also observed in the organisms of plant and fungi kingdoms, even in unicellular eukaryotes and prokaryotes [2,3,4]. However, many more cell death subtypes, as defined by cellular morphology, cell function and biochemical markers, had been identified in the past fifty years. Nomenclature Committee on Cell Death (NCCD) has named already twelve cell death forms with the canonical types of apoptosis, autophagy and necrosis among them, in 2018. As molecular cell biology, biochemistry, biomedicine and biology sciences keep developing, this research area continues expanding.

This review investigates the evolution of the scientific cell death concept and approaches to investigate it. The cell is programmed to die by many diverse mechanisms and subroutines. At the same time, understanding the interplay between life- and death-promoting signals, or more specifically – the mechanisms by which naturally-programmed cell death is induced or suppressed, may grant us the knowledge how to extend our lives. On one hand, hazardous environment causes chronic cell death that leads to organ malfunction; on the other hand, cellular life can be artificially prolonged. Moreover, progress is needed in dealing with immortal or cell death-resistant cells, e.g. in human cancers. As reviewed by Kaminskyy and Zhivotovsky [5], cell death can be pharmacologically targeted for the treatment of immunodeficiency, diabetes, atherosclerosis, ischemia, reperfusion injury,

infection, inflammation, autoimmune and neurological disorders, acute kidney injury and transplantation. However, the success is largely dependent on our understanding of what we know about a cell and what we still don't.

As cancer is expected to surpass cardiovascular disease as the leading cause of death in many high-income populations and become the disease No.1 [6], as well as the age-related diseases become usual in the aging society, concern in cell death regulation continues to grow. Paradoxically, when discussions about what is life continue, e.g. whether a virus is alive, an opposite thing – cell death – appeared to be equally important and not easy-going to define. A group of scientists who later established the committee called Nomenclature Committee on Cell Death (NCCD) put many efforts in distinguishing between live and dead at cellular level. Nevertheless, it became clear that a living cell is preloaded with explosives, i.e. suicidal molecules that are coded in our genome, and the abundance of those deadly molecules is amazing. Many different signal transducing proteins, proteases and channel components are present in the cytoplasm and in the plasma membrane of every single cell, counterbalanced by prosurvival molecular mechanisms [7]. It is really surprising why we are still alive.

## 2. THE 20<sup>TH</sup> CENTURY

In 1951, a scientist Glucksmann collected and documented over 70 scattered reports which had been published previously about cell deaths *in vivo* and *in vitro* [8]. This date may be considered as a starting point from which eukaryotic cell death science started evolving. Yet, there is data that cell death evidence may go back even into 19<sup>th</sup> century (the year 1842), as presented in one of the multiple chronologies of cell death [9]. As noted in the published analysis from the ISI-Science citation index [10] and nicely reviewed by Lockshin [11], the history of apoptosis, or a programmed cell death (PCD) to which this term had been applied for decades, made this field of research world-famous and fashionable. The number of publications has been growing enormously. Cell viability assays for *in vitro* evaluation of cytotoxicity were developing, but cellular morphology was the main criterion to describe the type of cell death while trying to fit into a container of three cell death types: apoptosis (regulated cell suicide; the hallmark – cell shrinkage, condensed and fragmented nucleus), autophagy (self cannibalism; the

hallmark – double-membrane vesicles in the cytoplasm) and necrosis (passive cell swelling; the hallmark – swelling mitochondria and increased cell size). Later, molecular patterns of a certain cell death type began to emerge. For example, ‘DNA-ladder’ as a result of internucleosomal DNA degradation, emergence of phosphatidylserine on the cell surface, and also activation of cysteine proteases caspases, were considered as obligate markers of apoptotic cell death. Some other immunohistochemical markers included cleaved cytokeratin-18, cleaved caspase-3, cleaved lamin A, phosphorylated histone H2AX, cleaved poly(ADP ribose) polymerase, and translocation of apoptosis-inducing factor AIF [12]. However, massive research of apoptosis led to inconsistency in the terminology, until a group of specialists decided to establish a committee which would become an authority. Thereafter, Nomenclature Committee on Cell Death published their first recommendations in 2005 [13], followed by publications in 2009 [14], 2012 [15], 2015 [16] and 2018 [4].

### 3. YEAR 2005

Briefly, in the article of 2005, all the known at that time cell death forms have been described, namely apoptosis, autophagic cell death, necrosis/oncosis, mitotic catastrophe, cornification, excitotoxicity, anoikis and Wallerian degeneration. Probably for the first time, a difference between ‘dying’ and ‘dead’ cells has been emphasized. According to suggested terminology, cell death was not as a process but rather a consequence *post factum*. Even in 2005 it was clear that there were atypical cell death forms that possessed the attributes of both apoptosis (active cell death) and necrosis (passive cell death). Moreover, it was apparent that there might be switching between different modes of cell death execution and that the definition of ‘point-of-no-return’ was extremely varied among different cells, thus the Committee chose to substantiate that the cell was ‘dead’ when the following criteria were met: i) its plasma membrane disintegrated, ii) the nucleus completely fragmented, iii) membrane-bound cell particles formed and engulfed by neighbour cells. Another important thing, the causes of cell death were imperatively appointed to be named in every case in biomedical research, especially the methods of active investigation, making a difference between death induction and death morphology. For example, ‘caspase-3-positive cells’ were to be more precise than ‘apoptotic

cells’, and ‘etoposide-induced cell death’ would not involve any disputes whether it is apoptotic, autophagic or necrotic cell death. Similarly, e.g. ‘TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP nick end labelling)-positive cells’ do not necessarily are dying, though it is presumed that they are; TUNEL assay simply detects DNA strand breaks, while in certain stem cells such DNA damage is slowly but successfully repaired [17]. Finally, cells with autophagic phenotype were suggested to be renamed as cells ‘with double-membrane vesicles’ or cells with ‘vesicular redistribution of LC3’, while autophagic cell death was questioned to exist at all [13].

Moreover, in 2005, NCCD questioned the usage of common pan-caspase inhibitor N-Benzyloxycarbonyl-Val-Ala-Asp fluoromethyl ketone (Z-VAD.fmk; with aspartyl residue either methylated or not). There were data that this inhibitor was non-selective towards caspases but also irreversibly inhibited cytoplasmic cysteine proteases calpains as well as lysosomal cysteine proteases cathepsins. In this regard, prevention of cell death by Z-VAD.fmk was suggested not to be called as ‘inhibition of caspase-dependent apoptosis’, as the above mentioned other proteases participate in various cell death events, including those of autophagy, necrosis and necroptosis, as later reviewed in [18] (Table 1).

Furthermore, in 2005, the Committee made a step towards combining several cell death modes (anoikis with apoptosis, oncosis with necrosis) and suggested refraining from the introduction of new terms like *aponecrosis* or *necroapoptosis*.

### 4. YEAR 2009

Later, in 2009, NCCD issued recommendations entitled ‘Classification of cell death: recommendations of the Nomenclature Committee on Cell Death 2009’. In this paper, several quite new atypical cell death forms were described on the basis of the published research. However, the main modalities of cell death were selected to be apoptosis, autophagy, cornification and necrosis. Probably because of this, the historical numeration (cell death type I, II or III) was proposed to be abandoned.

As in previous paper, NCCD continued to merge atypical death modalities with the main ones. As a consequence, mitotic catastrophe, anoikis and excitotoxicity have lost their autonomy, while

**Table 1. Functions of various cysteine proteases in cell death**

| Protein     | Functions   | Cell death modality   |
|-------------|---|---|
| Caspase-1   | Interleukin IL-1 $\beta$ and IL-18 conversion; Inflammation [4]     | Pyroptosis  |
| Caspase-2   | Sensing DNA damage [19]   | Apoptosis/ mitotic catastrophe  |
| Caspase-3   | Cleavage of multiple proteins, including activation of caspase-8/10 | Apoptosis [4]   |
| Caspase-8   | Activation of caspase-3; cleavage of Bid [16]                       | Extrinsic apoptosis (death receptors); Autophagic FADDosome [20]          |
| Caspase-9   | Activation of caspases-3/6/7  | Intrinsic apoptosis; Dependence receptor-induced extrinsic apoptosis [15] |
| Caspase-10  | FLIPosome formation; FADDosome formation; caspase-8 activation      | Necroptosis; Apoptosis [21]   |
| Caspase-12* | Effector of ER stress [22]; Antiinflammatory                        | Intrinsic apoptosis; Paraptosis   |
| Caspase-14  | Formation of epidermis [23]   | Cornification   |
| Cathepsins  | Proteolysis in lysosomes  | LDCD [4]; ADCD  |
| Calpains    | Proteolysis in cytoplasm **   | Necrosis; Ferroptosis; Apoptosis  |

\* Functional in rodents, but in majority of human population inactive due to a mutation [24]

\*\* Ca<sup>2+</sup>-dependent activation under Ca-overload conditions [25]

paraptosis, pyroptosis, pyronecrosis and entosis were left as an open question. Moreover, Wallerian degeneration was retracted from the cell death list due to the unfulfillment of criteria required for the definition of 'dead cell'. Specifically, peripheral neurons during Wallerian degeneration usually regenerate [14].

Importantly, NCCD found that morphological criteria were not sufficient to identify cell death type or modality; hence they suggested looking for biochemical and molecular markers specific to a certain demise of a cell. For example, implication of caspases, non-caspase proteases and Rip family proteins were proposed to be definitely important for this purpose in the future. And yes, they did.

## 5. YEAR 2012

In 2012, the third recommendation entitled 'Molecular definitions of cell death subroutines: recommendations of the Nomenclature Committee on Cell Death 2012' was published. NCCD kept their promise and discussed the pros and cons of both morphological and biochemical aspects of cell death. As declared in 2009, NCCD continued their mission to ensure uniformity in nomenclature and the use of accepted terminology and critical evaluation of new cell death modalities. Of note, the situation in laboratories had changed dramatically from 1970's to 2012, and although transmitted light

microscopes continued to be an obligate instrument in cell biology for the morphological evaluation of cell cultures, a bundle of molecular tools became available for such research. Moreover, well-defined molecular mechanism of classic apoptosis encouraged to look into the mechanisms of other cell death types. Albeit almost all atypical cell death forms were phenotypically intermediate between apoptotic and necrotic, they probably could have been quite well resolved and discriminated at the molecular level. Finally, novel biochemical tests were acquired for more convenient and quantitative patient diagnostics, thus historical cell death classification was reconsidered on the new basis.

In publication of 2012, many previously known molecular facts were accompanied with newly discovered cell signalling events and regulatory mechanisms which helped to better describe apoptosis, necrosis, autophagic cell death, anoikis, entosis, parthanatos, pyroptosis, netosis and cornification.

However, the Committee realized that cell viability methods were the weak part of the chain as still there was substantially no molecular indicator which would guarantee the exact answer about cell demise. It seemed that certain cell death markers played pleiotropic roles in physiological conditions as well as they were implicated in execution of different cell death

types. For example, caspase activation and phosphatidylserine exposure were not the unique features of apoptosis, not mentioning the intracellular level of ATP or ROS, and activity of reducing enzymes. In parallel, there were many quite different traditional cell viability assays: accumulation of specific dyes, release of intracellular proteins, glucose uptake, cell detachment, clonogenic, metabolism-based assays, TUNEL, BrdU or EdU incorporation, mitochondria membrane potential, calcium efflux into cytoplasm, Calcein-AM, total protein staining and similar [26]. Thereafter, it was absolutely necessary to recommend using more than one method for cell death quantification.

Nevertheless, very specific markers of cell death type or subtype began to emerge. In early 2000, ligand deprivation-induced dependence receptor signalling was discovered, and in 2012 NCCD added this type of cell death induction to the extrinsic apoptosis but as molecularly separate modality with involvement of caspase-9 instead of caspase-8. Similarly, intrinsic apoptosis was divided into caspase-dependent and caspase-independent. This cell death process was associated with generalized and irreversible mitochondria membrane potential dissipation, release of mitochondrial proteins into the cytosol or other sub-cellular compartments and inhibition of respiratory chain. Importantly, there was already enough proof that necrosis is a regulated process, thus terminology 'regulated necrosis' was introduced into the nomenclature. Similarly to earlier clarifications or certain terms associated with cell death, in the recommendations of 2012 NCCD named mitotic catastrophe as an 'onco-suppressive mechanism', not as cell death, as aberrant mitosis was proved to induce cell senescence in some cases [15].

## 6. YEAR 2015

As it was predicted, scientific perception about cell death has been evolving very rapidly in the past decade. The publication entitled 'Essential versus accessory aspects of cell death: recommendations of the NCCD 2015' did not disappoint in that sense. Just for to mention, NCCD publication of 2009 had 'only' 30 affiliations, followed by 46 affiliations in 2012, and listing 125 affiliations in 2015. Supposedly, there had to be major improvements in the nomenclature. And yes, it was.

Firstly, the article started with a confusing story about a giant mimivirus which could be infected

by other viruses. Such phenomenon has sparked the debates how to describe the differences between live and inert entities. What came second into the sight reading this recommendation, was the introduction of terms 'regulated cell death' and 'accidental cell death' (ACD), illustrated by a figure where ACD was a small object compared to RCD that contained the programmed cell death (PCD) in it. Further, the evidence that morphology of a dying cell was dynamic and dependent on genetic or pharmacological interventions was presented. In addition, the authors have summarized that usually there was no efficient cytoprotection beyond the hypothetical *point-of-no-return* in cell commitment. Subsequently, additional process of adaptation was introduced to precede cell death initiation, during which ATP and ROS levels oscillated in an anti-parallel manner as a consequence of RCD promoting and suppressing signalling. Hereafter, NCCD recommended to use the term '*initiation*' to indicate the RCD-causing events that were reversible due to still ongoing adaptive responses [16].

Another question exacerbated by NCCD in this publication was the role of damage-associated molecular patterns (DAMPs) in cell death induction. Briefly, certain molecules were identified to provoke specific reaction of the organism during which homing phagocytes were attracted to the DAMPs-releasing site and, more importantly, inflammation as well as DAMP-induced PCD was initiated through the activation of their receptors and signalling. Usually those molecules (now called alarmins) reside inside a cell; however, during infection or extreme non-physiological conditions they escape into extracellular medium as the plasma membrane of a cell ruptures. In the case of ACD, much higher levels of alarmins are released when compared to RCD. As summarized in Table 2, quite specific plasma membrane channels are intentionally formed (or activated) during regulated cell death for the controlled release of DAMPs.

The article ends with a stunning conclusion (quote): '*A growing body of data indicates indeed that the bona fide executioners of RCD, that is, the processes that directly drive cells across the boundary between life and death are less characterized, less inhibitable and perhaps more homogeneous than previously thought*'. Excitingly, a new term 'anastasy' was introduced to describe cellular function to recover from the late-stage death execution [31].

**Table 2. Channels in plasma membrane, responsible for cell death execution**

| <b>Protein</b>                         | <b>Activated by</b>                   | <b>Cell death modality</b>                         | <b>Notes</b>  |
|--|---------------------------------------|--|---|
| MLKL                                   | RIP3 (phosphorylation)                | Necroptosis  | MLKL octamer [27]   |
| DFNA5                                  | Caspase-3 (proteolysis)               | Secondary necrosis/ Apoptosis                      |   |
| Gasdermin D                            | Caspase-1/5 (proteolysis)             | Pyroptosis   |   |
| PANX1                                  | Caspase-3/7 (proteolysis)             | Apoptosis  |   |
| Connexins/ pannexins                   | N/A [28]                              | Apoptosis; Pyroptosis; Necrosis                    |   |
| NMDA channel                           | Glutamate/aspartate (opening)         | Excitotoxicity                                     | Excitotoxicity is considered as a form of ferroptosis in neural cells [4]                                 |
| Na <sup>+</sup> /K <sup>+</sup> ATPase | N/A [29]                              | Autosis/ Autophagic cell death                     | This ATPase is responsible for a large part of ATP consumption (>60% of cellular ATP in neurons) [29]     |
| Lipid peroxidation *                   | Fenton reaction                       | Ferroptosis  | * Non-specific leakage  |
| Perforin **                            | Physiological pH and Ca <sup>2+</sup> | Apoptosis (when in concert with granzyme protease) | ** Perforin and granzyme molecules are synthesized and secreted in granules by cytotoxic lymphocytes [30] |

In addition, based on 174 completely sequenced eukaryotic genomes, already in 2013 other authors postulated that ancestral eukaryotic cell (the progenitor of all eukaryotes) did not have the simplified version of cell death signalling pathways, but instead it was equally complex as that of the mammals today [32].

## 7. YEAR 2018

It was interesting for us, that in the publication of 2015 many forms of cell death were omitted and not discussed, perhaps reflecting the title of the article: 'essential vs. accessory'. Nevertheless, in their publication of 2009, cornification was one of the main forms of cell death, and quite distinct from others. Though it might be a bit confusing, the most recent recommendation of NCCD clarified the thing.

The article 'Molecular mechanisms of cell death: recommendations of the Nomenclature Committee on Cell Death 2018' was quite exceptional. The fact that it was accepted for publication in two days after submission definitely means a lot, together with 244 affiliations of the authors [4].

Briefly, major cell death subroutines were summarized there: intrinsic apoptosis, extrinsic apoptosis, mitochondrial permeability transition (MPT)-driven necrosis, necroptosis, ferroptosis, pyroptosis, parthanatos, entotic cell death, netotic cell death, lysosome-dependent cell death, autophagy-dependent cell death, immunogenic cell death. Importantly, the diagram presented in the article suggests that every of the mentioned cell death modalities interplays with a neighbour one and the transitions are possible in the sequence as listed here, connecting immunogenic cell death with intrinsic apoptosis to close the circle of death [4]. Beside, the full set of cell death-related terminology was described in an explaining manner in one sentence, along with detailed revision of published data. Every newly systematized cell death form was extensively covered in the recommendation – over a thousand of references have been used in this paper. Definitely, the recommendation of 2018 should be referred as the most reliable and complete document generalizing the cell death science. Here, in Table 3, current cell death modalities are described.

For example, previously undiscerned mode called lysosome-dependent cell death (LDCD) was described as a type of regulated cell death demarcated by primary lysosomal membrane

permeabilization and precipitated by cathepsins, with optional involvement of mitochondrial outer membrane permeabilization and caspases. It is a bit confusing as lysosomes were discovered in late 1950's, and already in 1960's cytolytic enzymes have been demonstrated to play a role in programmed cell death [47]. Autophagy is also dependent on lysosomes, but additional and separate cell death modality – LDCD – which is implicated in inflammation, tissue remodelling (e.g., mammary gland involution after lactation), aging, neurodegeneration, cardiovascular disorders, intracellular pathogen response, as well as in physiological elimination of a fraction of emerging male germ cells, was a surprise.

As mentioned above, since 2015, cornification was retracted from the list of cell death modes. Instead of naming it a 'cell death' subtype, with an exceptional involvement of caspase-14 in the fate of keratinocytes, NCCD re-qualified this process as 'terminal differentiation' because dead corneocytes were neither disposed off nor phagocytised, but became an integral part of an organism and continued serving a function. Interestingly, the surface of plants is covered with dead cells that grant the organism protection from harsh environment conditions including sun radiation [2]. In NCCD nomenclature, cell senescence, mitotic catastrophe and cornification are sub-grouped under a category of 'non-lethal processes'. Alternatively, neural cell death upon over-stimulation with neurotoxic amino acids (glutamate and aspartate), previously known as oxitosis or excitotoxicity, recently has been assigned to ferroptosis. Indeed, it is known that iron is accumulated in the brain where it is under a risk to catalyze the Fenton reaction in the presence of hydrogen peroxide [48]. The latter in turn accumulates when glutathione concentration drops as a result of glutamate-dependent inhibition of the  $C_x^-$  system (cystine-glutamate antiporter) [4].

However, NCCD has repeated many times, that the field is constantly evolving, and that the nomenclature may be reconsidered. E.g., recent publication draws a connection of autophagy with entosis (cell cannibalism) through a shared molecular mechanism involving TM9SF4, mTORC and AMPK proteins [33]. We can recall and repeatedly emphasize that autophagy and entosis are defined as non-lethal processes, unless they culminate in cell death. Hence the correct names for cell demise are 'entotic cell death' and 'autophagy-dependent cell death' (ADCD) [4].

**Table 3. Cell death modes according to NCCD 2018 [4]**

| <b>Cell death modality</b>                                  | <b>Brief description</b>  | <b>References</b> |
|---|---|-------------------|
| Autophagy-dependent cell death                              | A form of RCD that mechanistically depends on the pro-survival autophagic machinery (or components thereof). Autosis is a specific instance of ADCD that critically relies on the plasma membrane Na <sup>+</sup> /K <sup>+</sup> -ATPase.  | [33][34]          |
| Entotic cell death  | A type of RCD that originates from actomyosin-dependent cell-in-cell internalization (entosis) by non-phagocytic cells and is executed by lysosomes.  | [35]              |
| Extrinsic apoptosis   | Specific variant of RCD initiated by perturbations of the extracellular microenvironment detected by plasma membrane death or dependence receptors, propagated by CASP8 and executed mainly by CASP3.   | [36]              |
| Ferroptosis   | A form of RCD initiated by oxidative perturbations inside a cell, susceptible to inhibition by iron chelators and lipophilic antioxidants, and under constitutive control by glutathione peroxidase GPX4.   | [37]              |
| Immunogenic cell death                                      | A form of RCD that is sufficient to activate an adaptive immune response to viral infection in immunocompetent hosts. It is mediated by DAMP release.   | [38]              |
| Intrinsic apoptosis   | Type of RCD initiated by perturbations of the extracellular or intracellular microenvironment, demarcated by mitochondrial outer membrane permeabilization (with implication of BH3 domain proteins), and precipitated by executioner caspases, mainly CASP3. Plasma membrane integrity in vivo is retained through the process. A specific variant of intrinsic apoptosis elicited by the loss of integrin-dependent attachment to the extracellular matrix is known as anoikis. | [39][40]          |
| Lysosome-dependent cell death                               | A type of RCD demarcated by primary lysosome membrane permeabilization and precipitated by cathepsins, with optional involvement of mitochondrial outer membrane permeabilization and caspases.   | [41]              |
| Mitochondrial permeability transition (MPT)-driven necrosis | RCD triggered by perturbations of the intracellular microenvironment (severe oxidative stress and Ca overload) and relying on peptidylprolyl isomerase F.   | [42]              |
| Necroptosis   | A modality of RCD triggered by perturbations of extracellular or intracellular homeostasis that critically depends on MLKL, RIPK3, and (at least in some settings) on the kinase activity of RIPK1.   | [43]              |
| NETotic cell death  | A ROS-dependent modality of RCD restricted to cells of hematopoietic derivation, intended for pathogen neutralization and associated with neutrophil extracellular traps (NET) extrusion.   | [44]              |
| Parthanatos   | A modality of RCD initiated by PARP1 hyperactivation and precipitated by the consequent bioenergetic catastrophe coupled to AIF-dependent and MIF-dependent DNA degradation.  | [45]              |
| Pyroptosis  | A type of RCD that critically depends on the formation of plasma membrane pores by members of the gasdermin protein family, often as a consequence of inflammatory caspase (CASP1) activation in response to pathogen invasion.   | [46]              |



## 8. PERSPECTIVES

It becomes clear that mandatory component of life is the biological barrier, i.e. the plasma membrane and the regulating molecules which support its integrity. Therefore, a eukaryotic cell may be called 'dead' when its plasma membrane loses integrity and continuously permits uncontrollable flux of ions as well as larger than usual molecules. However, it is still too far from the final answer how to control it in pathological conditions.

The field of cell death types, forms or modalities continues developing and may grant us major surprises in the future. For example, a new role for a well-known apoptosis-inducing protease caspase-8 has been discovered. It appears that caspase-8 is active in certain living cells, negatively regulates a lytic form of cell death necroptosis, participates in the cleavage of inflammatory interleukin-1 $\beta$  to its mature bioactive form, and regulates cytokine transcription [49]. Furthermore, in 2018, some authors have introduced a new name – oxeiptosis – to describe a novel cell death pathway which was independent of caspases, initiated by oxygen radicals and different from those of ROS-induced apoptosis, necroptosis and ferroptosis. This discovery is important as it has identified a new ROS-sensing molecular switch – signalling molecule KEAP1 which leads to activation of AIFM1 (Apoptosis-Inducing Factor 1 Mitochondrial) and starts with oxidation of cysteines in C-terminus of KEAP1 [50]. Alternatively, the associations between apoptosis, autophagy and regulated necrosis have been discovered [51], compromising the pioneer three-type classification of cell death described in [1], and perhaps similar findings in the future may have an impact on upcoming NCCD recommendations.

In addition, recent publication of Seehawer et al. may start a new page in our knowledge about cancer, namely how neighbouring cells epigenetically react to different cell death modalities in the vicinity. The authors discovered that certain drugs (HDTV and Epo) induced different cell death types in mouse liver and also resulted in different expression of cytokine mRNAs. Depending on that, different types of liver cancer – hepatocellular carcinoma or intrahepatic cholangiocarcinoma – developed in mosaic mouse models [52]. The findings described in the paper bring additional complexity to cancer progression, at the same

time they shed some light on fundamental aspects of cell behaviour.

## 9. CONCLUSION

Generally, there should be ways to overcome cancer cell resistance to RCD induction by initiating other cell death modes which probably are suppressed less than other within the malignant cell. Alternatively, neoplastic cells may be guided to terminally differentiate and thereby stop growing as a tumour. However, we have to realize that there are more than 20.000 genes in the human genome and only less than a half of them are recognized in performing a known biological function. Moreover, the genes are regulated epigenetically and the majority of genes produce alternatively-processed proteins which in turn may have pleiotropic functions during different developmental stages of a cell life and death.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCE

1. Schweichel JU, Merker HJ. The morphology of various types of cell death in prenatal tissues. *Teratology*. 1973;7(3): 253–66.
2. Greenberg JT. Programmed cell death: A way of life for plants. *Proc. Natl. Acad. Sci. USA*. 1996;93:12094–12097.
3. Kurusu T, Kuchitsu K. Autophagy, programmed cell death and reactive oxygen species in sexual reproduction in plants. *J. Plant Res*. 2017;130(3):491–499.
4. Galluzzi L, Vitale I, Aaronson SA, Abrams JM, Adam D, Agostinis P, Alnemri ES, Altucci L, Amelio I, Andrews DW, Annicchiarico-Petruzzelli M, et al. Molecular mechanisms of cell death: Recommendations of the Nomenclature Committee on Cell Death; 2018. *Cell Death Differ*; 2018.
5. Kaminsky VO, Zhivotovsky B. Cell death-based treatment of various diseases: A

- fifty-year journey. *Cell Death Dis.* 2018; 9(2):110.
6. Cao B, Bray F, Beltrán-Sánchez H, Ginsburg O, Soneji S, Soerjomataram I. Benchmarking life expectancy and cancer mortality: Global comparison with cardiovascular disease 1981-2010 j2765. *BMJ.* 2017;357.
  7. Portt L, Norman G, Clapp C, Greenwood M, Greenwood MT. Anti-apoptosis and cell survival: A review. *Biochim. Biophys. Acta - Mol. Cell Res.* 2011;1813(1):238–259.
  8. Glucksmann A. Cell deaths in normal vertebrate ontogeny. *Biol. Rev. Camb. Philos. Soc.* 1951;26(1):59–86.
  9. Häcker G, Vaux DL. A chronology of cell death. *Apoptosis.* 1997;2(3):247–56.
  10. Garfield E, Melino G. The growth of the cell death field: An analysis from the ISI-Science citation index. *Cell Death Differ.* 1997;4(5):352–61.
  11. Lockshin RA. Programmed cell death 50 (and beyond). *Cell Death Differ.* 2016; 23(1):10-17.
  12. Holubec H, Payne CM, Bernstein H, Dvorakova K, et al. Assessment of apoptosis by immunohistochemical markers compared to cellular morphology in ex vivo-stressed colonic mucosa. *J. Histochem. Cytochem.* 2005;53(2):229-235.
  13. Kroemer G, El-Deiry WS, Golstein P, Peter ME, Vaux D, Vandenabeele P, et al. Classification of cell death: Recommendations of the Nomenclature committee on cell death. *Cell Death Differ.* 2005;12: 1463-1467.
  14. Kroemer G, Galluzzi L, Vandenabeele P, Abrams J, Alnemri ES, et al. Nomenclature Committee on Cell Death 2009, Classification of cell death: Recommendations of the Nomenclature Committee on Cell Death 2009. *Cell Death Differ.* 2009; 16(1)3–11.
  15. Galluzzi L, Vitale I, Abrams JM, Alnemri ES, Baehrecke EH, Blagosklonny MV, Dawson TM, Dawson VL, El-Deiry WS, Fulda S, et al. Molecular definitions of cell death subroutines: Recommendations of the Nomenclature committee on cell death 2012. *Cell Death Differ.* 2012;19(1):107–120.
  16. Galluzzi L, Bravo-San Pedro JM, Vitale I, Aaronson SA, Abrams JM, et al. Essential versus accessory aspects of cell death: Recommendations of the NCCD 2015. *Cell Death Differ.* 2015;22(1):58–73.
  17. Schneider L. Survival of neural stem cells undergoing DNA damage-induced astrocytic differentiation in self-renewal-promoting conditions *in vitro*. *PLoS One.* 2014; 9(1):e87228.
  18. Radogna F, Dicato M, Diederich M. Cancer-type-specific crosstalk between autophagy, necroptosis and apoptosis as a pharmacological target. *Biochem. Pharmacol.* 2015;94(1):1–11.
  19. O'Byrne KJ, Richard DJ. Nucleolar caspase-2: Protecting us from DNA damage. *J. Cell Biol.* 2017;216(6):1521–1523.
  20. Bell BD, Leverrier S, Weist BM, Newton RH, Arechiga AF, Luhrs KA, Morrisette NS, Walsh CM. FADD and caspase-8 control the outcome of autophagic signaling in proliferating T cells. *Proc. Natl. Acad. Sci. U.S.A.* 2008;105(43):16677.
  21. Mohr A, Deedigan L, Jencz S, Mehrabadi Y, Houlden L, Albarenque SM, Zwacka RM. Caspase-10: A molecular switch from cell-autonomous apoptosis to communal cell death in response to chemotherapeutic drug treatment. *Cell Death Differ.* 2018; 25(2):340–352.
  22. Hitomi J, Katayama T, Taniguchi M, Honda A, Imaizumi K, Tohyama M. Apoptosis induced by endoplasmic reticulum stress depends on activation of caspase-3 via caspase-12. *Neurosci. Lett.* 2004;357(2): 127-130.
  23. Denecker G, Ovaere P, Vandenabeele P, Declercq W. Caspase-14 reveals its secrets. *J. Cell Biol.* 2008;180(3):451–458.
  24. Ferwerda B, McCall MBB, de Vries MC, Hopman J, Maiga B, et al. Caspase-12 and the Inflammatory Response to *Yersinia pestis*. *PLoS One.* 2009;4(9):e6870.
  25. Bano D, Ankarcrona M. Beyond the critical point: An overview of excitotoxicity, calcium overload and the downstream consequences. *Neuroscience Letters;* 2018.
  26. Kalvelytė AV, Imbrasaitė A, Krestnikova N, Stulpinas A. Adult Stem Cells and Anticancer Therapy. In *Advances in Molecular Toxicology;* 2017.
  27. Huang D, Zheng X, Wang Z, Chen X, et al. The MLKL channel in necroptosis is an octamer formed by tetramers in a dyadic process. *Mol. Cell. Biol.* 2017;37(5): e00497-16.
  28. Gilleron J, Carette D, Segretain D, Pointis G. Multiple and complex influences of connexins and pannexins on cell death.

- Biochim. Biophys. Acta - Biomembr. 2018; 1860(1):182–191.
29. Muñoz-Pinedo C, Martin SJ. Autosis: A new addition to the cell death Tower of Babel. *Cell Death Dis.* 2014;5(7):e1319.
  30. Spicer BA, Conroy PJ, Law RH, Voskoboinik I, Whisstock JC. Perforin—A key (shaped) weapon in the immunological arsenal. *Semin. Cell Dev. Biol.* 2017; 72:117–123.
  31. Tang HL, Tang HM, Hardwick JM, Fung MC. Strategies for tracking anastasis, a cell survival phenomenon that reverses apoptosis. *J. Vis. Exp.* 2015;96.
  32. Zmasek CM, Godzik A. Evolution of the animal apoptosis network. *Cold Spring Harb. Perspect. Biol.* 2013;5(3):a008649–a008649.
  33. Fais S, Overholtzer M. Cell-in-cell phenomena, cannibalism, and autophagy: Is there a relationship? *Cell Death Dis.* 2018;9(2):95.
  34. Liu Y, Shoji-Kawata S, Sumpter RM, Wei Y, Ginet V, Zhang L, Posner B, Tran KA, et al. Autosis is a Na<sup>+</sup>,K<sup>+</sup>-ATPase-regulated form of cell death triggered by autophagy-inducing peptides, starvation, and hypoxia-ischemia. *Proc. Natl. Acad. Sci.* 2013; 110(51):20364–20371.
  35. Florey O, Kim S, Overholtzer M. Entosis: Cell-in-cell formation that kills through entotic cell death. *Curr. Mol. Med.*; 2015.
  36. Brenner D, Blaser H, Mak TW. Regulation of tumour necrosis factor signalling: Live or let die. *Nat. Rev. Immunol.* 2015.
  37. Yang WS, Stockwell BR. Ferroptosis: Death by lipid peroxidation. *Trends in Cell Biology.* 2016.
  38. Kepp O, Tartour E, Vitale I, Vacchelli E, et al. Consensus guidelines for the detection of immunogenic cell death. *Oncolmmunology.* 2014.
  39. Green DR, Oguin TH, Martinez J. The clearance of dying cells: Table for two. *Cell Death and Differentiation.* 2016.
  40. Alanko J, Mai A, Jacquemet G, Schauer K, Kaukonen R, Saari M, Goud B, Ivaska J. Integrin endosomal signalling suppresses anoikis. *Nat. Cell Biol.* 2015.
  41. Serrano-Puebla A, Boya P. Lysosomal membrane permeabilization in cell death: New evidence and implications for health and disease. *Ann. N. Y. Acad. Sci.* 2016.
  42. Vanden Berghe T, Linkermann A, Jouan-Lanhouet S, Walczak H, Vandenabeele P. Regulated necrosis: The expanding network of non-apoptotic cell death pathways. *Nature Reviews Molecular Cell Biology.* 2014.
  43. Kaiser WJ, Sridharan H, Huang C, et al. Toll-like receptor 3-mediated necrosis via TRIF, RIP3, and MLKL. *J. Biol. Chem.* 2013.
  44. Brinkmann V, Reichard U, Goosmann C, et al. Neutrophil Extracellular Traps Kill Bacteria. *Science (80-);* 2004.
  45. David KK. Parthanatos, a messenger of death. *Front. Biosci.* 2009.
  46. Jorgensen I, Miao EA. Pyroptotic cell death defends against intracellular pathogens. *Immunological Reviews.* 2015.
  47. Lockshin RA, Williams CM. Programmed cell death. V. Cytolytic enzymes in relation to the breakdown of the intersegmental muscles of silkworms. *J. Insect Physiol.* 1965;11(7):831–44.
  48. Tower J. Programmed cell death in aging. *Ageing Res. Rev.* 2015;23:90–100.
  49. Feltham R, Vince JE, Lawlor KE. Caspase-8: not so silently deadly. *Clin. Transl. Immunol.* 2017;6(1):e124.
  50. Scaturro P, Pichlmair A. Oxeiptosis—a cell death pathway to mitigate damage caused by radicals. *Cell Death Differ.* 2018; 25(7):1191–1193.
  51. Chen Q, Kang J, Fu C. The independence of and associations among apoptosis, autophagy, and necrosis. *Signal Transduction and Targeted Therapy.* 2018.
  52. Seehawer M, Heinzmann F, D'Artista L, Harbig J, et al. Necroptosis micro-environment directs lineage commitment in liver cancer. *Nature.* 2018.

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