MOLECULAR MECHANISMS OF CELL DEATH:
IMPLICATIONS FOR DISEASE PATHOGENESIS

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CHAPTER 1

APOPTOSIS: MECHANISMS, REGULATION, AND BIOLOGICAL SIGNIFICANCE

Yalda HEKMATSHOAR [1]
Aynur KARADAĞ GÜREL [2]

INTRODUCTION

The survival and proper functioning of multicellular organisms rely on the tightly controlled process of cell death. This regulation maintains tissue balance by coordinating the creation of new cells with the removal of old or damaged ones. Cell death is essential for regular tissue renewal, defense against infections, and developmental processes like embryogenesis and metamorphosis. The two major forms of cell death are programmed cell death (apoptosis) and necrosis (1).

Apoptosis is a strictly regulated, evolutionarily conserved process that allows cells to self-eliminate in a methodical manner without causing inflammation or damaging neighboring cells (2). Unlike necrosis, which is uncontrollable and often accompanied by swelling and membrane rupture, apoptosis involves the deliberate breakdown of cellular components (2). Tissue homeostasis, development, and defense against damaged or potentially cancerous cells all depend on it (1). In 1972, Kerr and associates provided a clear description of apoptosis, defining its morphological characteristics and differentiating it from necrosis (3).

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CHAPTER 2

NETOSIS: PROGRAMMED CELL DEATH MEDIATED BY EXTRACELLULAR TRAPS

Seda TAŞIR [1]

INTRODUCTION

Neutrophils can neutralize pathogens not only through classical mechanisms such as phagocytosis and degranulation, but also via DNA structures released into the extracellular environment. Neutrophil Extracellular Traps (NETs) are recognized as one of the rapid and effective defense mechanisms of the innate immune system. NETs are three-dimensional networks composed of DNA fibers embedded with granule-derived antimicrobial proteins, histones, and proinflammatory damage-associated molecular pattern (DAMP) molecules. This organization plays a critical role in both pathogen capture and the orchestration of immune responses. Although NET formation was initially thought to be exclusively dependent on cell death, current evidence indicates that different signaling pathways can mediate NET release while preserving cell viability. Consequently, NETosis can be classified into multiple subtypes depending on the triggering factors, molecular processes, and cellular outcomes. Moreover, various disease conditions can significantly shape the protein composition of NETs.

NEUTROPHIL DEFENSE STRATEGIES AND NETOSIS DEVELOPMENT

Neutrophils are the most abundant and rapid effector cells of the innate immune system, playing a central role in protecting hosts against pathogens (1). Despite their short lifespan, neutrophils constitute approximately 50–70% of all circulating leukocytes in humans. They originate from hematopoietic stem cells in the

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CONCLUSION

Although neutrophil extracellular traps (NETs) are one of the immune system's powerful defense mechanisms against infections, uncontrolled or excessive NET formation can play a harmful role in numerous pathophysiological processes. The literature indicates that nuclear DNA decondensation, the release of granular and cytoplasmic proteins into the extracellular space, ROS production, and the presence of various stimuli are key determinants in NET formation. The activation of lytic and non-lytic NETosis pathways by different triggers indicates that the process is regulated by a context-dependent, complex network. Current findings demonstrate that NETs are not only crucial for infection control but also serve as key regulators in a wide range of pathological conditions, including autoinflammatory diseases, thrombosis, and malignancies. Therefore, understanding the molecular dynamics of NET formation and developing therapeutic strategies targeting NET components hold the potential to open new treatment avenues across a wide range of diseases.

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CHAPTER 3

PYROPTOSIS: CELLULAR SIGNALING OF INFLAMMATORY CELL DEATH

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PYROPTOSIS: AN INTRODUCTION AND BRIEF HISTORICAL OVERVIEW

In multicellular organisms, maintaining a balance between cell proliferation and cell death is critical for ensuring physiological homeostasis. Unlike accidental cell death, numerous distinct forms of programmed cell death (PCD) have now been identified in cells (1).

Pyroptosis is an immunogenic form of PCD, characterized by unique cell swelling, osmotic lysis, and plasma membrane rupture. This process results in the release of pro-inflammatory intracellular components into the extracellular space, serving as a powerful alarm signal to the immune system (2). The idea of pyroptosis began in the 1990s with observations like the death of macrophages infected with *Shigella flexneri* (2). However, researchers clarified the concept around 2000 when they described a specific type of cell death in Salmonella-infected macrophages that was morphologically different from apoptosis (3,4). In 2001, the term "pyroptosis" was officially used to describe a Caspase-1-dependent process that, although it shares some features with apoptosis, is mainly driven by inflammatory cascades (2). An important turning point in understanding its mechanism came with the identification of Gasdermin D as the key executioner protein. The molecular basis for the observed rupture of the plasma membrane

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inhibition prevents the release of mature IL-1β and IL-18, which are key actors in inflammation. This helps to decrease the detrimental consequences of pyroptosis in a variety of disorders (2).

Instead of directly inhibiting targets, additional therapeutic approaches focus on upstream factors that influence the assembly or stability of inflammasome components. Metformin, for example, stimulates autophagy and inhibits NLRP3-mediated pyroptosis via activating the AMPK/mTOR pathway. Meanwhile, irisin and mitochondrial ubiquitin ligase activation can prevent GSDMD-mediated pyroptosis (2). Furthermore, several nanobodies have been developed to selectively target the N-terminal domain of GSDMD, reducing its pore-forming activity and thus decreasing pyroptotic cell death (46). The mentioned therapeutic approaches address a variety of targets, such as indirect modulation via pathways like AMPK/mTOR activation (2) and direct suppression of inflammasome components such NLRP3, GSDMD, and caspases (47).

These various strategies suggest that pyroptosis can be targeted in a variety of inflammatory diseases, especially those associated with an uncontrolled innate immune response (47). A complete understanding of these inhibitory processes is crucial for developing targeted therapeutics for diseases characterized by excessive or inappropriate pyroptotic activity, such as intervertebral disc degeneration (48) and malignancies (2).

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CHAPTER 4

CUPROPTOSIS: MECHANISMS OF COPPER-INDUCED CELL DEATH

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INTRODUCTION

Programmed cell death (PCD) processes are vital for maintaining homeostasis and preventing cancer. In addition to well-characterized pathways such as apoptosis, autophagy, and necroptosis, new metal-dependent PCDs such as ferroptosis have recently been discovered. In 2022, Tsvetkov and colleagues elucidated "cuproptosis," a distinctive mode of cellular demise initiated by the direct interaction of copper (Cu) ions with lipoylated proteins within the mitochondrial respiratory chain. This chapter details the molecular mechanism of cuproptosis, its potential physiological roles in normal cells, and how it is disrupted in cancer cells. In particular, we highlight the differences in sensitivity of cancer cells with increased copper levels and mitochondrial metabolism to this pathway. We also talk about how targeting cuproptosis could be a new way to treat tumors that don't respond to standard treatments and the new ways that tumors can become resistant. The integration of cuproptosis into cancer biology provides a new framework for therapeutic development.

Cell death biology has experienced a significant paradigm shift in recent years. In addition to the traditional pathways of apoptosis, necrosis, and autophagy, many new programmed cell death (PCD) mechanisms have been found. These include ferroptosis, which is caused by iron-dependent lipid peroxidation;

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structure and metabolic pathways, impairs mitochondrial respiration, and overwhelms detoxification systems. As oxidative stress accumulates, mitochondrial membrane integrity collapses, proteotoxic stress intensifies, and key metabolic enzymes undergo pathological acylation and oligomerization—driving cells toward cuproptosis. Collectively, these findings underscore that copper is both vital and potentially lethal, and that the cell's ability to precisely regulate copper uptake, utilization, sequestration, and export ultimately determines whether copper acts as a life-sustaining cofactor or a catalyst of regulated cell death.

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CHAPTER 5

NECROPTOSIS: CONTROLLED NECROSIS AND INFLAMMATION

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DEFINITION AND HISTORY OF NECROPTOSIS

Necroptosis is a regulated form of cell death that exhibits the morphological characteristics of necrosis. It leads to cell and organelle swelling, disruption to the integrity of the plasma membrane and the release of intracellular contents. This triggers a strong inflammatory response in the microenvironment. Unlike apoptosis, necroptosis is a caspase-independent cell death pathway. This type of cell death occurs between apoptosis and accidental necrosis (1, 2). Functionally, it plays a crucial role in a wide range of physiological and pathological processes including defence against viral infections, inflammatory regulation, ischaemic tissue injury and tumour biology (1). The rupture of the plasma membrane during necroptosis releases intracellular molecules act as damage-associated molecular patterns (DAMPs) that activating immune responses (3).

Regulation or control of necrosis was first proposed in the early 2000s, based on evidence that showed tumour necrosis factor-α (TNF-α) could cause cell death despite the presence of pharmacological inhibitors of caspase activity (4, 5). This discovery undermined the long-held perspective that necrosis was entirely random and uncontrolled. In 2005, Degterev et al. introduced the term 'necroptosis' to formally describe this regulated caspase-independent process (6). The prevailing view today is that necroptosis is a form of programmed

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Necroptosis is considered a highly immunogenic form of cell death because the release of damage-associated molecular patterns (DAMPs) following membrane rupture attracts and activates dendritic cells. Activated dendritic cells subsequently present tumour antigens to naïve $CD8^+$ T cells, promoting their differentiation into cytotoxic effector cells and contributing to an immunogenic tumour microenvironment. These findings support the hypothesis that inducing necroptosis in apoptosis-resistant tumour cells may be a promising therapeutic strategy. However, necroptosis can also promote tumour progression. DAMPs released from necroptotic cells may support angiogenesis, cell proliferation, immune evasion, and metastasis in certain cancers. Epigenetic silencing of RIPK3 through promoter hypermethylation such as that induced by the oncometabolite 2-hydroxyglutarate (2-HG) in IDH1-mutant tumours has been shown to confer resistance to necroptosis. Similarly, RIPK3 promoter hypermethylation may contribute to RIPK3 loss in human small cell lung cancer. In colorectal cancer, METTL3-mediated enhancement of TRAF5 m6A modification in M2-polarized tumour-associated macrophages impairs necroptosis and has been linked to the development of chemoresistance to oxaliplatin. The RNA-editing enzyme ADAR1 has recently emerged as a key mediator of resistance to immune checkpoint blockade therapy. ADAR1 inhibits antitumour immunity by suppressing immunogenic double-stranded RNAs. Its depletion or mutation results in Z-RNA accumulation (ZBP1 activation) and subsequent RIPK3-mediated necroptosis, restoring responsiveness to immunotherapy (57).

Despite its antitumor potential, necroptosis can also have detrimental effects in certain circumstances by promoting uncontrolled cell proliferation, inflammation, and metastasis. Therefore, depending on the tumor type and microenvironment, activation or inhibition of necroptosis may provide therapeutic benefit. In particular, inhibition of necroptotic signaling has been proposed as a strategy to reprogram the tumor microenvironment by enhancing highly immunogenic myeloid and T-cell responses (57).

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CHAPTER 6

FERROPTOSIS

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Programmed cell death (PCD) is an essential physiological process that contributes to cellular homeostasis, embryogenesis, tissue development, immune regulation and aging (1–3). Over the past decades, several types of cell death, like apoptosis, autophagy and pyroptosis have widely been studied for diverse physiological and pathophysiological conditions (4). In addition to those cell death mechanisms, a type of regulated cell death has newly characterized—ferroptosis, which is distinct from these classical pathways.

Ferroptosis was discovered in 2003 by Dolma et al. who identified erastin, a compound that caused death in RAS-mutant cancer cells without showing the hallmark features of apoptosis or necrosis, including chromatin condensation, caspase activation or mitochondrial cytochrome c release (5). Later research showed that iron chelators could inhibit this distinct type of cell death through iron-dependent mechanisms (6,7). In 2012, Dixon et al. officially introduced the term "ferroptosis" to describe an iron-dependent and non-apoptotic form of cell death characterized by the accumulation of lipid reactive oxygen species (ROS). This process represents a unique type of oxidative cell death, primarily driven by excessive lipid peroxidation and an inadequate antioxidant capacity to remove lipid peroxides (7).

Ferroptosis is different from other types of cell death by specific morphological changes in mitochondria such as decreased or missing cristae, increased

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such as drug specificity, off-target effects and the complex relationship between ferroptosis and other regulated cell death pathways must carefully be addressed. Even though research on ferroptosis is expanding, the majority of findings are mostly derived from *in vitro* and *in vivo* animal studies, with limited clinical data accessible.

To sum up, ferroptosis is a complicated and important biological process that involves metabolism, oxidative stress and cell death. Moreover, its involvement in the progression of various diseases represents a new approach of treatment by targeting its regulatory networks. Therefore, developing effective strategies could pave the way for innovative therapies for such as cancer, neurodegenerative disorders, ischemia-reperfusion and acute kidney injury.

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CHAPTER 7

AUTOPHAGY AT THE CROSSROADS OF CELL SURVIVAL AND CELL DEATH

Nevin BELDER[1]

INTRODUCTION

Autophagy broadly defines an evolutionarily conserved set of catabolic pathways that are critically important for sustaining cellular homeostasis. This essential process culminates in the transport of intracellular cargo, including macromolecules like proteins and membrane-bound organelles, to the lysosome for degradation and subsequent recycling. Operating at a basal level in all cell types, autophagy prevents the detrimental accumulation of damaged proteins and compromised organelles. This mechanism therefore assumes a fundamental role in the quality control of cytoplasmic constituents, thereby underpinning the maintenance of cellular equilibrium (1,2)

Life is sustained by an ongoing balance between the creation and breakdown of the cell's own components, a principle that becomes clearer as our understanding of cellular physiology expands. Proteins, membranes and entire organelles are continually renewed, and this constant turnover distinguishes living cells from engineered systems that rely on fixed parts. To maintain this internal balance, cells draw steadily on nutrients and energy from their surroundings. In animals, these demands are met largely through the digestion of dietary proteins, which provides both the building blocks and the metabolic fuel required to support cellular renewal (3). The understanding of intracellular protein degradation mechanisms began with the discovery of the lysosome as a distinct cellular structure by the Belgian cytologist Christian de Duve in 1955 (Figure 1). De Duve was awarded the 1974 Nobel Prize in Physiology or Medicine for his pioneering work on this

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preserving its homeostatic roles. As mechanistic insight continues to converge with disease-focused research, autophagy is likely to become increasingly central to diagnostic, prognostic and therapeutic strategies. Its still-expanding biology positions autophagy as a key axis in future efforts to understand and treat human disease.

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CHAPTER 8

MITOPHAGY: THE PROCESS OF FINE-TUNING MITOCHONDRIAL AND CELLULAR HOMEOSTASIS

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INTRODUCTION

Mitochondria are double membrane-bound organelles possessing their own genetic material (mtDNA) as well as ribosomes, and a cytosolic environment to carry out the reactions related to energy metabolism, biogenesis of certain metabolites, regulation of metabolism, thermogenesis, signaling, and apoptosis. Although the discovery of mitochondria dates back to the mid-1800s, their pivotal function in energy metabolism was demonstrated by Kennedy and Lehninger much later in 1950 (1, 2). Possessing all four conserved features of the living organisms is the core of the endosymbiotic theory, which states that an archaeon engulfed an α-proteobacterium, triggering a mutualistic relationship so efficient that they thrived together around 2 billion years ago (3). Now, we know the mitochondria are not just the powerhouse of the cell, but they are essential to a wide variety of different functions, including metabolic processing and signaling. Therefore, comprehending the mitochondrial dynamics in detail is crucial to establishing and maintaining cellular balance ultimately.

The eminence of the mitochondria lies in their role in cellular homeostasis. In order to maintain cellular homeostasis, the cell must institute mitochondrial homeostasis, the organelle-level balance of mitochondrial activity. Mitochondrial dynamics are governed through controlling its population by mitochondrial biogenesis, organelle degradation, and fission/fusion processes (4). Moreover, the maintenance of mitochondrial proteostasis and mtDNA needs to be addressed

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to modulate the process to take control of disease progression. Although the full content of mitophagy is yet to be elucidated, developing inhibitors and activators of mitophagy is a promising strategy for pharmacological achievement. The knowledge we gather on mitophagy and its associated metabolism will increase drastically throughout the 'omics' technology (genomics, transcriptomics, proteomics, metabolomics, etc.). Hopefully, any information assists us in exploiting the central hub of mitochondrial quality for control of a broad spectrum of benefits, including disease-specific therapeutics, modifying pathophysiological conditions, and even reversing the impact of aging on cells.

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CHAPTER 9

THE ROLE OF CELL DEATH IN CANCER AND THERAPEUTIC TARGETS

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INTRODUCTION

Cancer remains one of the primary global health challenges due to its complex biological structure and its capacity to adapt to and develop resistance against conventional treatments. The current understanding of cancer biology has moved beyond characterising the disease solely as uncontrolled cell proliferation; it has begun to emphasise the central role of disrupted programmed cell death mechanisms in tumour development, progression, and resistance to treatment (1,2). Cellular homeostasis requires maintaining a delicate balance between cell proliferation and cell death, but in malignant tumours, this balance is disrupted, and the suppression of cell death leads to a proliferative advantage.

Apoptosis, long considered the sole programmed cell death mechanism, is one of the fundamental processes that prevents tumour development by eliminating cells carrying DNA damage or oncogenic mutations. However, cancer cells can evade apoptosis through mechanisms such as the overexpression of anti-apoptotic proteins, the inactivation of tumour suppressor genes (e.g., TP53), and the suppression of caspase activity, thereby supporting the survival and progression of malignant cells (3,4). This situation has led to the acceptance of resistance to cell death as one of the fundamental distinguishing characteristics of cancer.

In recent years, the identification of alternative forms of programmed cell death, such as necroptosis, ferroptosis, pyroptosis, and autophagy-related cell

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CHAPTER 10

INBORN ERRORS OF IMMUNITY IN APOPTOSIS AND AUTOPHAGY

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INTRODUCTION AND DEFINITION OF INBORN ERRORS OF IMMUNITY

Primary immunodeficiencies are monogenic disorders that are seen due to gene defects that disrupt immune system function with variable clinical phenotypes. Although the term **"inborn errors of immunity" (IEI)** has become more widely used in recent literature (1), the term PIDs will be utilised throughout this section. PIDs represent a heterogeneous group of disorders, often presenting in early childhood, and may be accompanied by autoimmune, autoinflammatory, allergic diseases, and malignancies. They can be inherited in autosomal dominant, autosomal recessive, or X-linked patterns, and are characterized by quantitative or functional deficiencies of immune system components, leading to significant morbidity and mortality. According to the 2024 update from the International Union of Immunological Societies (IUIS), 508 different gene mutations have been linked to PIDs, which are categorized into 10 groups based on clinical and laboratory features (2) **(Table 1)**, each containing several subgroups. The prevalence of PIDs varies globally, ranging from 1/10,000 to 1/100,000 in general populations (4), whereas in countries with a high rate of consanguinity, such as Türkiye, the prevalence is approximately 30.5/100,000 (3). In the Middle East, similar prevalence rates ranging from 0.8 to 30.5/100,000 have been reported.

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