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Reaching the point-of-no-return: The cornerstone of glioblastoma treatment?

Montse Alemany, Jordi Bruna, and Victor J. Yuste

All author affiliations are listed at the end of the article

Corresponding Authors: Jordi Bruna, Neuro-Oncology Unit, Hospital Universitari de Bellvitge-ICO L'Hospitalet (IDIBELL), Feixa Llarga s/n Street, 089098 Barcelona, Spain (35078jbe@comb.cat); Victor J. Yuste, Cell Death, Senescence and Survival Group, Departament de Bioquímica i Biologia Molecular and Institut de Neurociències, Facultat de Medicina, Universitat Autònoma de Barcelona, Campus de Bellaterra, Can Domenech Street, M Building, 08193 Bellaterra, Spain (victor.yuste@uab.cat).

Abstract

The activation of cellular death programs does not necessarily predetermine an inevitable outcome. Identifying the precise moment when a cell irreversibly transitions from life to death presents a significant challenge in its assessment and measurement. In this review, we explore the critical alterations in cellular structures that have been proposed as the *point-of-no-return*. Using glioblastoma as a model—one of the most aggressive and lethal tumor types with a remarkable ability to evade cell death—we highlight the challenge of reaching the *point-of-no-return*. Glioblastoma cells often exhibit impaired function of the apoptotic endonuclease, DFF40/CAD/CPAN, leading to incomplete apoptosis and genomic instability. The sublethal activation of DFF40/CAD/CPAN not only allows tumor cells to survive but can also drive more aggressive phenotypes and enhance therapeutic resistance. We underscore the need to reassess glioblastoma treatment strategies from broad cytotoxic approaches to more targeted therapies that exploit specific vulnerabilities within regulated cell death (RCD) pathways.

Key Points

- The hallmark that best identifies the *point-of-no-return* in cell death is nuclear fragmentation.
- DFF40/CAD/CPAN endonuclease is impaired in glioblastoma, representing a limiting step in achieving nuclear fragmentation.
- Current therapeutic strategies show limited efficacy in pushing glioblastoma cells beyond the threshold of irreversible cell death.

Balancing Life or Death Decisions

Multicellular organisms rely on a tightly regulated interplay between cellular proliferation and cell death to maintain homeostasis.[1] Disruption of this equilibrium can lead to different pathological conditions. For example, excessive cell death contributes to neurodegenerative diseases, while uncontrolled proliferation drives cancer and autoimmune disorders.[2] Though often perceived as opposing cellular processes, cell death and proliferation exhibit intricate crosstalk, ensuring proper organismal function. Early observations by Vogt (1842) on toad metamorphosis provided the first glimpse into this concept.[3] Further evidence was provided in 1966 by Saunders and Fallon, who observed a process of cellular demise during the formation of free digits in vertebrate animals.[4] Wright and colleagues provided more recent evidence in 1983, describing neuronal cell death during the early embryonic stages of the mammalian nervous system development.[5] The available evidence unequivocally demonstrates that cell death is an

indispensable biological process for the proper functioning and survival of multicellular organisms.[6]

In this sense, tumors recapitulate certain features of multicellular organisms; however, they do so through a dysregulated balance between cellular proliferation and death. Moreover, tumor cells exhibit a remarkable capacity for dynamic adaptation, enabling them to thrive in their altered microenvironment.[7] This phenotypic plasticity, while facilitating rapid responses to environmental fluctuations, also render them more vulnerable to death under certain conditions.[8] This increased vulnerability likely stems from the ongoing remodeling of the tumor cell's genome, potentially accumulating deleterious mutations that compromise cellular fitness and heighten sensitivity to cytotoxic stress.[9]

Paradoxically, although tumor cells exhibit inherent vulnerabilities compared to healthy cells, most current nonsurgical therapies demonstrate limited efficacy. This discrepancy highlights a critical question: what are the key mechanisms by which tumor cells evade therapy-induced cell death? An accurate answer requires acknowledging that the fundamental imperative of an individual cell is the preservation of homeostasis and the activation of adaptative mechanisms to safeguard its survival under fluctuating physiological or pathological conditions. This principle applies to both tumor and nontumor cells and is achieved through diverse cellular adaptation pathways to induced damage.

To develop an effective antitumor therapy, it is crucial to guide cells towards a point at which survival mechanisms are irreversibly overcome, and cells can no longer recover vital functions. The establishment of this critical threshold, often referred to as the *point-of-no-return* (Figure 1), remains a significant challenge for the scientific community.[8] Several factors may influence cell's progression towards this point. These include both extracellular and intracellular determinants. The extracellular milieu consists of noncellular components (eg, drugs, extracellular matrix, soluble proteins) as well as cellular elements, such as constitutive and nonconstitutive neighboring cells. Conversely, intracellular determinants encompass the intricate network of mechanisms governing the metabolic status of the cell at a given time. Considering these determinants, we would like to focus on a new perspective: the limited effectiveness of existing treatments to drive tumor cells to a state of irreversible death, the *point-of-no-return*. Addressing this gap is essential for the development of treatments capable of exploiting tumor vulnerabilities, overcoming cellular resistance, and ultimately achieving durable therapeutic success.

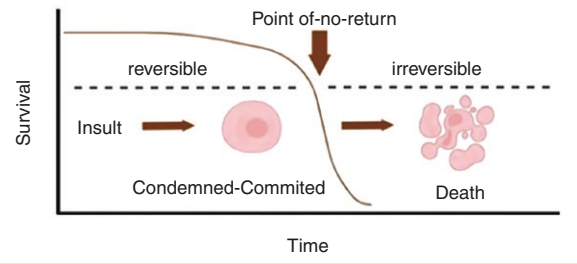

Figure 1. The *point-of-no-return* in cell death. Following a lethal insult, the cell transitions into a state of commitment to death. While this stage may remain reversible if the stimulus is withdrawn, passing the point-of-no-return renders the process irreversible, culminating in cell death. Created in BioRender. Velasco, R. (2025) https://BioRender.com/1uoaovn.

Cell Death and the *Point-of-No-Return*

Pinpointing the exact nature of cell death and developing a foolproof method to identify it has been a huge challenge for the scientific community. Except under abrupt and unexpected conditions induced by extreme high-intensity physical or chemical stimuli, cell death constitutes a highly conserved and orchestrated biological process, typically executed with precision.[9] In fact, cellular machinery involved in cell death is conserved across a broad spectrum of organisms, including multicellular and unicellular eukaryotes, as well as certain prokaryotes.[10] Schrödinger proposed in 1944 that the demise of living systems, including individual cells, can be defined in physical terms as the inability to maintain energetic gradients.[11] Building upon this, he theorized that biological systems counteract entropy by converting environmental energy into internal order. This implies that life is contingent upon the acquisition and efficient utilization of energy. As open systems, living organisms preserve their structure and prevent deterioration by maintaining a continuous influx of energy. More recently, John Garland has taken this line of thought a step further by offering a thermodynamic perspective on neoplastic transformation. He suggests that malignancy reflects not merely the accumulation of genetic mutations, but a fundamental shift in cellular energy dynamics. According to this view, cancer cells enhance entropy production (the dissipation of energy) through dysregulated processes such as uncontrolled proliferation, enhanced motility, and loss of structural integrity. Introducing the concept of "fractal entropy," Garland proposes that oncogenic alterations stabilize dynamic, energy-dissipating network patterns, thereby promoting the persistent, disordered behavior characteristic of tumor cells.[12]

Nevertheless, despite the physical perspective, identifying the precise moment of irreversible transition from life to death in normal or neoplastic cells remains challenging. What are the key mechanisms and cellular processes that cause a cell to cross the threshold between life and irreversible death? Can a cell with impaired energy gradient maintenance reverse the process of cell death and restore normal function? If recovery is possible, how much damage must accumulate, or how long must elapse before the cell's demise becomes unavoidable? Current knowledge is insufficient to definitively answer these questions, resulting in a lack of consensus within the scientific community regarding the precise identification of the *point-of-no-return* in cell death.

Potential and Proposed Points-of-No-Return in Cell Death

The definition of the *point-of-no-return* in cell death remains imprecise and lacks consensus among researchers.

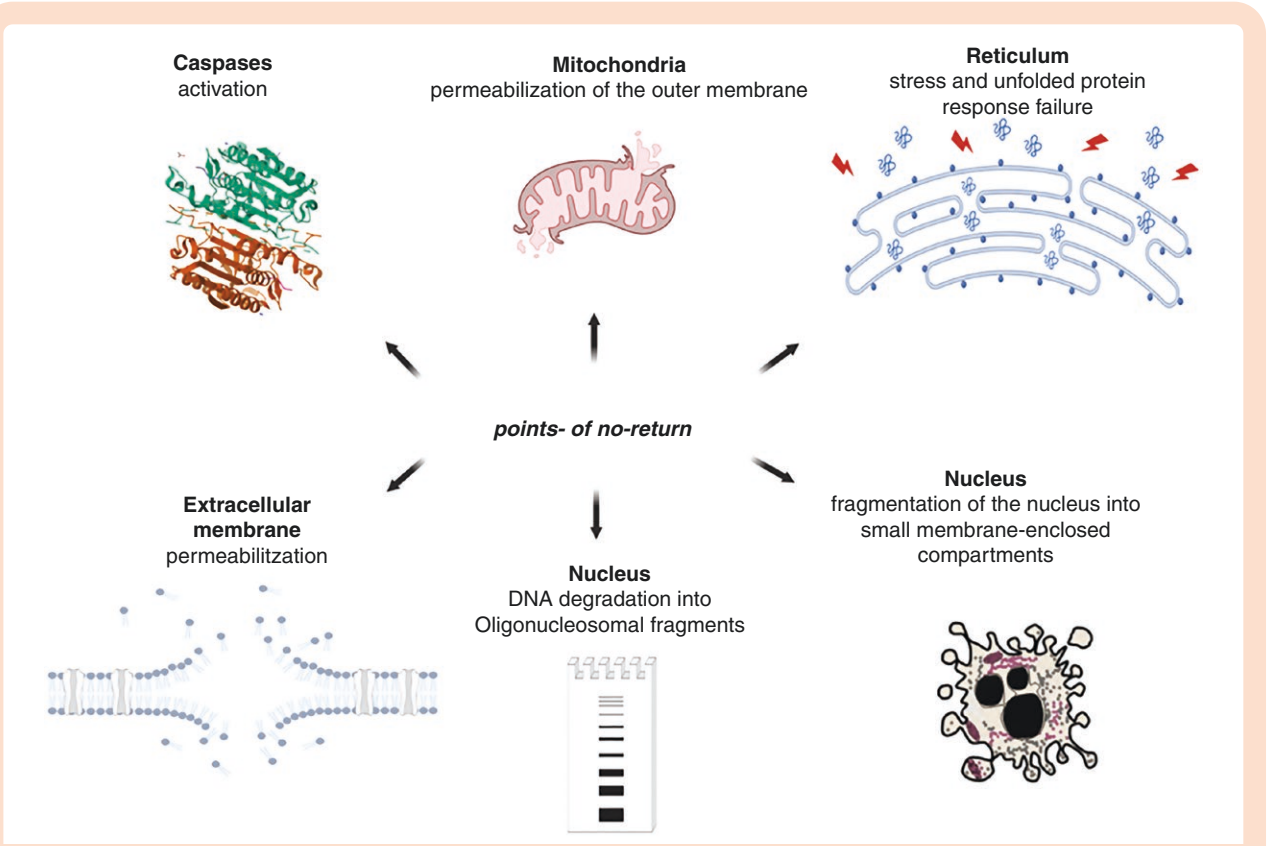

Figure 2. Schematic representation of the proposed mechanism underlying *points-of-no-return.* Created in BioRender. Velasco, R. (2025) https://BioRender.com/7kg2dyo.

This concept is closely associated with the initiation of different RCDs pathways and their associated biochemical cascades. These intracellular processes are influenced by a complex interplay of dynamic factors—such as stimulus intensity, resource availability, and the cell's overall energy state—making it difficult to precisely delineate a *point-of-no-return*. Some researchers propose that irreversible damage to critical cellular structures defines this threshold, offering a basis for a more unified definition. This perspective also accounts for the simultaneous contributions of multiple RCDs pathways and biochemical processes to cell death. In the following sections, we will examine the proposed molecular and cellular checkpoints involved in the irreversibility of cell death (Figure 2).

The Activation of Caspases

Caspase (acronym for "cysteine-dependent aspartate-specific protease") activation was proposed as one of the first irreversible events, marking a *point-of-no-return* for the cell, even in the absence of the death-inducing stimulus. Caspases, synthesized as catalytically inactive zymogens,[13] are a family of proteases essential for apoptosis or inflammation.[14,15] Apoptosis, a form of programmed cell death, is initiated by a death stimulus that activates a proteolytic cascade involving caspases.[16] This process results in characteristic morphological changes, including cell shrinkage, DNA fragmentation, nuclear condensation and fragmentation, and the formation of apoptotic bodies.[17,18] While caspase activation is a critical step in the execution of apoptosis, it is not necessarily an irreversible *point-of-no-return*. Although caspase activation has been proposed as a key molecular marker of commitment to cell death,[19,20] evidence indicates that inhibiting caspase activity can, in certain circumstances, rescue cells from apoptosis.[21] Such inhibition can be categorized as either exogenous or endogenous. Exogenous inhibitors include small compounds such as Q-VD-OPh, z-VAD-fmk, and IETD-fmk, which directly inhibit caspase activity.[22] Endogenous mechanisms, on the other hand, involve cellular proteins that regulate apoptotic pathways either by directly inhibiting caspase activity or by modulating upstream apoptotic signaling.

The first group of endogenous inhibitors consists of proteins that directly target caspases to block their proteolytic activity. These proteins are crucial in preventing caspase activation or inactivating already active caspases. Notable examples include the inhibitor of apoptosis proteins (IAPs), such as XIAP, cIAP1, and cIAP2,[23] the FLICE-inhibitory protein (FLIP),[24] and members of the serpin family, like Serpin B9.[25]

The second group encompasses proteins that regulate apoptotic signaling upstream of caspase activation. These proteins act by either preventing the release of pro-apoptotic factors from mitochondria or by modulating signaling pathways that promote apoptosis. The Bcl-2

family of proteins, including Bcl-2 and Bcl-X_L,[26] play critical roles in maintaining mitochondrial integrity and preventing cytochrome c release. Additionally, heat shock proteins (HSPs), such as HSP27 and HSP70, contribute to cellular survival by stabilizing proteins and mitigating apoptotic stress.[27] Akt/PKB (Protein Kinase B) further modulates apoptosis by phosphorylating and inactivating pro-apoptotic proteins, like Bad, thereby preventing the downstream apoptotic cascade.[28]

Since the activation of the caspase system failed to provide conclusive evidence to definitively establish it as the *point-of-no-return* in apoptosis, attention shifted toward exploring structural and functional cellular alterations.

Mitochondria: Permeabilization of the Outer Membrane

Mitochondrial outer membrane permeabilization (MOMP) is a critical event often viewed as the *point-of-no-return* in cell death. Triggered by various pathways, including apoptosis, ferroptosis, necroptosis, pyroptosis, and parthanatos, MOMP compromises the mitochondrial outer membrane. This disruption leads to decreased ATP production, dissipation of the mitochondrial membrane potential, uncoupling of the respiratory chain, and increased reactive oxygen species generation. Additionally, MOMP releases proteins like cytochrome c and Smac/Diablo from the mitochondrial intermembrane space into the cytosol, disrupting cellular metabolism and initiating apoptosis by activating caspases or neutralizing their inhibitors.[29,30]

Given mitochondria's vital role in cell survival, MOMP has been considered the irreversible commitment point to cell death.[31] However, recent findings indicate that cells can recover even after MOMP,[32,33] if the stressor is insufficiently intense or of limited duration, thus failing to reach the threshold required to trigger the irreversible commitment to cell death.

While the mechanisms safeguarding mitochondrial outer membrane integrity remain unclear,[34] some mitochondria can retain their integrity post-MOMP. Although cells must manage the consequences of MOMP-released products for successful recovery,[35] the occurrence of cell death without MOMP[19] suggests that MOMP may not be the definitive point-of-no-return.

The Reticulum: Stress and Unfolded Protein Response Failure

The endoplasmic reticulum (ER) is an intricate cellular organelle essential for maintaining cellular homeostasis. Its main functions encompass protein synthesis, folding, modification, and quality control, alongside lipid biosynthesis and calcium storage. A delicate balance within the ER is crucial for optimal cellular function. ER homeostasis disruptions, often induced by factors such as increased protein load, oxidative stress, or calcium imbalance, can trigger the unfolded protein response (UPR). This adaptive signaling pathway restores ER equilibrium by enhancing protein folding capacity, reducing protein synthesis, and promoting the degradation of misfolded proteins. However, when ER stress becomes chronic and the UPR is overwhelmed, the accumulation of misfolded proteins can lead to ER dysfunction and ultimately, cell death, which can occur through multiple pathways, including apoptosis, autophagy, necroptosis, pyroptosis, and ferroptosis. $Ca2^+$ release from the ER[36] and caspase-mediated processing of IRE1 (an ER transmembrane receptor)[37] are proposed as potential irreversible triggers for the transition from cell survival to death. Nevertheless, attempts to confirm these mechanisms as cell death commitments have yielded inconclusive results.[38,39] Therefore, we can assume that the point-of-no-return in ER stress-induced cell death is likely a complex interplay of multiple factors, including the intensity and duration of the stressor. As a result, ER stress alone cannot be definitively identified as a singular, irreversible trigger of cell death.

Extracellular Membrane Permeabilization: Pore-Forming Proteins as Drivers of Plasma Membrane Destabilization

In recent years, scientists have identified new forms of RCD beyond apoptosis.[40,41] Notably, necroptosis (triggered by the activation of several members of the TNF-related death receptors) and pyroptosis (triggered by pro-inflammatory signals)[42] involve the activation of pore-forming proteins, namely MLKL and Gasdermin D (GSDMD), respectively. This leads to the loss of plasma membrane integrity, resulting in cell death.[43] As a result, plasma membrane perforation has been proposed as the *point-of-no-return* in these other forms of RCD. However, several studies have demonstrated that the Endosomal Sorting Complexes Required for Transport machinery can mediate plasma membrane repair by counteracting the activity of MLKL[44] and GSDMD,[45] thereby ensuring cell survival.[46,47]

Nucleus: DNA Degradation into Oligonucleosomal Fragments

The integrity of DNA is essential for cellular viability, as DNA damage inherently limits the life span of the cell. For this reason, DNA damage has been proposed as the critical point-of-no-return in cell death.

Among the various forms of cell death, apoptosis is uniquely characterized by its direct impact on DNA integrity. During apoptosis, the cell activates a cascade of signaling pathways culminating in the activation of endonucleases responsible for DNA breakdown.[48] This fragmentation, known as oligonucleosomal or internucleosomal fragmentation, occurs through the selective targeting of DNA regions between nucleosomes by endonucleases, resulting in precise DNA cleavage. Among these, DFF40 (also called CAD or CPAN) is the only endonuclease directly activated by caspases, and it plays a pivotal role in executing the characteristic DNA fragmentation observed during apoptosis.[49] DFF40/CAD/CPAN facilitates two distinct forms of DNA damage: single-stranded breaks, which progress to double-stranded breaks (DSBs).[50] These double-stranded DNA fragments typically manifest as a characteristic "ladder-like pattern" when visualized using electrophoresis

on a neutral agarose gel.[51] While oligonucleosomal DNA degradation with DSBs is considered the most detrimental form of DNA damage, recovery is possible, albeit more challenging, through DNA repair mechanisms.[52]

Nucleus: Fragmentation of the Nucleus into Small Membrane-Enclosed Compartments

Nuclear fragmentation, or karyorrhexis, is a hallmark of apoptosis that occurs alongside oligonucleosomal DNA degradation[17] and is also orchestrated by the endonuclease DFF40/CAD/CPAN. This event is irreversible, as a fragmented nucleus can no longer sustain the cellular machinery and structures required to maintain the energy network patterns and gradients essential for life. It represents a critical tipping point, beyond which cell death becomes inevitable.[53]

The mechanisms discussed earlier have the potential to drive a cell to irreversible death if activated on a massive scale, suggesting they could also become *points-of-no-return*. However, a significant challenge—one that is rarely assessable—is determining the minimum threshold beyond which a cellular process transitions into an irreversible state of terminal decline or death. Among these, nuclear fragmentation (Figure 3) constitutes a definitive *point-of-no-return*, unequivocally indicating irreversible cell death, and can be detected by reliable and easy-to-perform methods.[55]

A Molecular Link in the *Point-of-No-Return*: The Apoptotic Endonuclease DFF40/CAD/CPAN

Oligonucleosomal DNA degradation and nuclear fragmentation are hallmarks of apoptotic cell death, mediated by the endonuclease DNA Fragmentation Factor 40 kDa subunit (DFF40), also known as Caspase-Activated DNase (CAD) and CasPase-Activated Nuclease (CPAN).[56–58] This endonuclease specifically cleaves double-stranded DNA (dsDNA) and is inhibited by various noncleavable substrates, including single-stranded DNA, single- and double-stranded RNA, and RNA-DNA hybrids.[59] In proliferating cells, DFF40/CAD/CPAN forms a complex with its inhibitor ICAD$_L$ (Inhibitor of CAD), also known as DFF45.[56,57,60] ICAD$_L$ contains two caspase cleavage sites at aspartic acid residues 117 and 224.[56,60] Beyond its inhibitory function, ICAD$_L$ also works as a molecular chaperone, preventing CAD aggregation.[61–65]

The hypothesis that DFF40/CAD/CPAN serves as the endonuclease responsible for key biochemical features of apoptosis, such as oligonucleosomal DNA degradation and nuclear fragmentation, emerged from observations of its expression in cells undergoing nuclear disassembly following apoptotic stimuli, but not in cells lacking this process. This hypothesis was further validated by studies in CAD-null mice, while viable, failed to exhibit the typical apoptotic biochemical hallmarks.[66] DFF40/CAD/CPAN selectively cleaves chromatin at internucleosomal linker DNA regions. Its homodimeric structure, featuring two active sites that bind to the DNA minor groove in a "scissors-like" conformation,[67] facilitates cleavage of the phosphodiester backbone, resulting in DSBs.[59,68] Conserved histidine residues within the active site are essential for nuclease activity.[69] Additionally, DFF40/CAD/CPAN preferentially targets exposed regions of naked DNA with a dyad axis of symmetry, based on pyrimidine and purine content.[70] Regarding DFF40/CAD/CPAN regulation, several negative regulators have been identified beyond DFF45/ICAD$_L$. These include divalent metal ions, such as $Cu2^+$ or $Zn2^{+}$,[71] anionic polymers (RNA, poly-glutamic acid, and heparin),[72] as well as regulatory proteins such as CAD Inhibitor that Interacts with ASK1 (CIIA),[73] and the nucleolar protein Nucleophosmin (NPM)/B23.[74] Conversely, positive regulators include $Mg2^+$, ionic strength (50–125 mM K^+), basic pH,[75] co-activators such as Histone H1, High Mobility Group Box 1 and 2 (HMGB 1/2), Topoisomerase II,[76] and phosphorylated H2AX[77] (Figure 4).

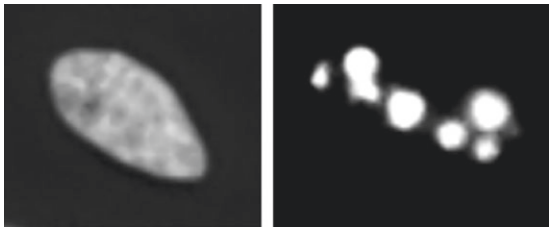

Figure 3. Representative fluorescence microscopy images of nuclei in SH-SY5Y human neuroblastoma-derived cells, either untreated or treated with 1 μM staurosporine for 24 h. Nuclear morphology was assessed by Hoechst 33258 staining. Treated cells display nuclear fragmentation, a classical hallmark of apoptosis. The SH-SY5Y cell line was chosen due to its well-established use as a reproducible in vitro model for studying apoptotic nuclear changes.[54]

Glioblastoma Cells: A Highly Resistant Model for Nuclear Fragmentation

Glioblastoma, among the most aggressive known tumors, exemplifies a scenario in which conventional therapeutic strategies consistently fail to achieve complete eradication of residual tumor cells.

The current standard treatment for newly diagnosed glioblastoma consists of maximal safety surgical resection, followed by radiotherapy combined with concomitant chemotherapy (temozolomide), and subsequent adjuvant temozolomide administration.[78,79] Despite the intensity and combination of therapeutic approaches, as well as advances in basic research, glioblastoma remains an incurable disease. Relapse or progression occurs in nearly all patients (>99%). Nowadays, individuals diagnosed with glioblastoma face a median overall survival of 15–16 months following diagnosis with the current treatments.

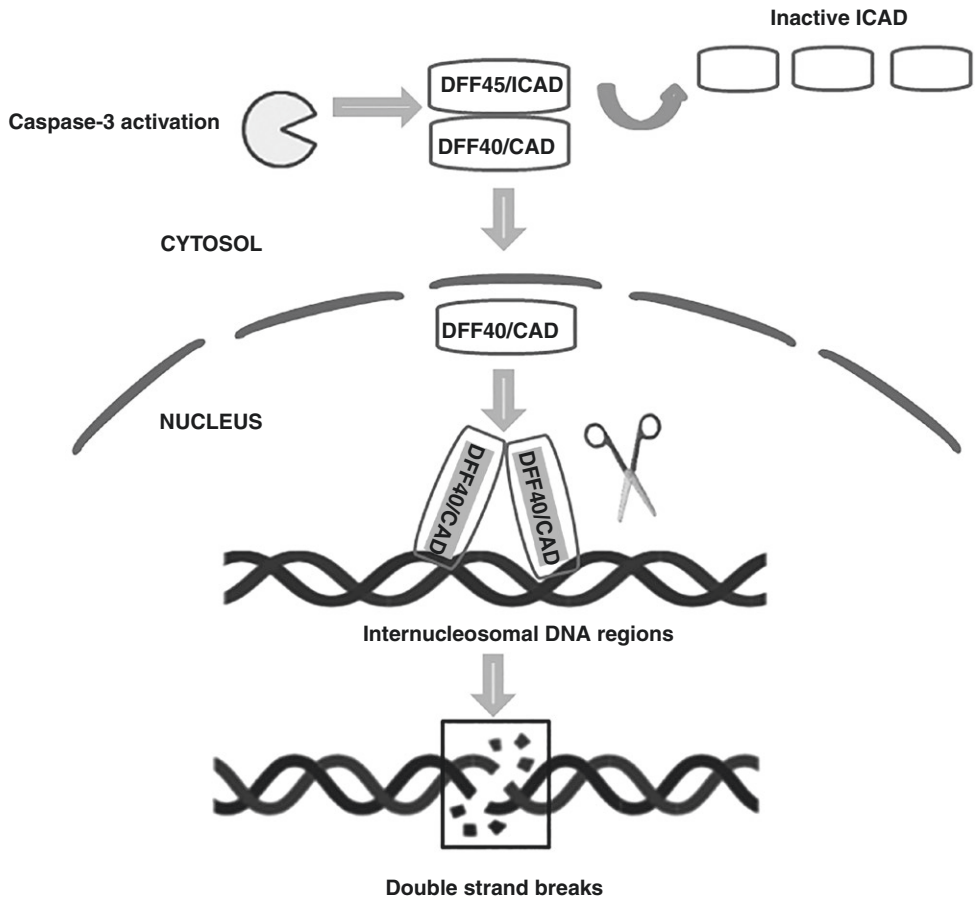

Figure 4. Diagram of activation of the endonuclease DFF40/CAD/CPAN. Once caspase-3 is activated, it cleaves DFF45/ICAD, thereby releasing DFF40/CAD/CPAN from its inhibitory complex and enabling the formation of DFF40/CAD/CPAN homodimers. The homodimers, which function analogously to molecular scissors, induce widespread double-stranded DNA breaks, ultimately leading to chromatin degradation and nuclear fragmentation. Created in BioRender. Velasco, R. (2025) https://BioRender.com/8xubpbc.

Since the publication of positive results from the randomized phase III clinical trial in 2005,[78] subsequent phase III trials investigating alternative agents have failed to demonstrate superior efficacy. Emerging systemic approaches, including small molecule inhibitors, immunotherapy (such as mRNA vaccines and CAR-T cell therapy), immunomodulators, and virotherapy represent innovative strategies currently under investigation. However, these efforts have either not resulted in clinically meaningful and substantial improvements in patients' survival compared to the established standard of care or remain in the preliminary phases of clinical development.[80]

Special mention should be made to the EF-14 study, which evaluated Tumor Treating Fields (TTFs), as the only phase III clinical trial since 2005 to demonstrate improved survival rates in glioblastoma patients.[81] TTFs is a noninvasive anticancer therapy that uses low-intensity, alternating electric fields delivered through the skin of the scalp, to disrupt tumor cell division and interfere with DNA repair mechanisms.[82] Nevertheless, the current standard of care, whether combined with TTFs or not, continues to offer only limited clinical benefits.

Consequences of Incomplete Apoptosis in Glioblastoma: Sublethal Activation of DFF40/CAD/CPAN and Genomic Instability

One factor limiting the effectiveness of conventional therapies for glioblastoma is the resistance of glioblastoma cells to undergo complete apoptotic cell death following exposure to cytotoxic agents.[83,84] These cells often fail to display apoptotic hallmarks (oligonucleosomal DNA degradation and nuclear fragmentation). This resistance can be attributed to several factors: (1) insufficient caspase levels; (2) normal caspase levels with inadequate activation; (3) sufficient caspase activation but insufficient levels of DFF40/CAD/CPAN; or (4) mislocalization of DFF40/CAD/CPAN activation, preventing it from occurring in the appropriate cellular compartment, such as the cytosol. Each of these factors can disrupt the apoptotic process, potentially leading to genomic instability[85] and contributing to a more aggressive tumor phenotype. Clinically, this may manifest as tumor progression or recurrence with increased

aggressiveness. In this context, impaired apoptosis results in inadequate nuclear processing, which can trigger local inflammation.[86] These alterations in the tumor microenvironment could compromise the efficacy of several therapies, including immunotherapies.

Assisting Glioblastoma Cells in Reaching the *Point-of-No-Return*

A focus on the concept of the *point-of-no-return* in cell death pathways reveals that multiple critical mechanisms governing programmed cell death and stress–response mechanisms are profoundly disrupted in glioblastoma. These tumors exhibit dysfunction across all key checkpoints associated with irreversible cell death. The caspase system, particularly the executioner caspases, caspase-3 and -7, is often defective, compromising the effective execution of the final steps of apoptosis.[87] Similarly, MOMP is frequently impaired, largely due to the overexpression of antiapoptotic Bcl-2 family members and recurrent TP53 mutations, which together inhibit the activation of pro-apoptotic effectors.[88] Moreover, UPR is constitutively and maladaptively activated in glioblastoma. Dysregulation of the PERK, IRE1α, and ATF6 signaling branches contributes to enhanced tumor cell survival under conditions of ER stress.[89] Epigenetic mechanisms and posttranslational modifications can contribute to alterations in pore-forming proteins such as GSDMD, impairing plasma membrane rupture and consequently reducing susceptibility to membrane-disruptive forms of RCD.[90] Particularly relevant are the abnormalities in nuclear events—specifically oligonucleosomal DNA degradation and nuclear fragmentation—both critically dependent on DFF40/CAD/CPAN endonuclease activity. These nuclear processes constitute terminal and irreversible steps in the execution of cell death, and their dysfunction may represent a major obstacle to effective therapeutic response in glioblastoma.

Traditionally, cancer treatments, excluding immunotherapy, have primarily aimed to induce cell death by activating executioner caspases through various mechanisms. The pro-apoptotic stimuli generated by these therapies are diverse and depend on the drug class, treatment modalities (eg, chemotherapy or radiotherapy), and their underlying mechanisms of action. However, this therapeutic strategy faces several significant challenges. First, systemic treatments must achieve adequate bioavailability across all cancer cells while avoiding unacceptable toxic side effects and overcome natural or tumor-induced barriers, such as the blood–brain barrier and hypoxic tumor regions. Second, overcoming various cellular resistance mechanisms, including DNA repair, redundant growth factor signaling pathways, and alternative metabolic adaptations, is essential. Finally, even after circumventing all these physical and cellular challenges and correctly activating the caspase proteolytic system, glioblastoma cells still face an unresolved molecular barrier that hinders the proper activation of DFF40/CAD/CPAN.[51,84,91]

Considering the current clinical outcomes observed in glioblastoma patients, it might be advisable to expand research efforts towards strategies that effectively restore or enhance DFF40/CAD/CPAN activation. This is critical for achieving complete cell death by reaching the *point-of-no-return*, namely, inducing nuclear fragmentation. In this context, our research has demonstrated that, despite deficient DFF40/CAD/CPAN protein expression in human glioblastoma cells, these cells can still exhibit apoptotic nuclear hallmarks under appropriate stimuli. Specifically, we showed that gossypol, a derivative of the cotton plant, can successfully activate DFF40/CAD/CPAN and complete the apoptotic program.[91] However, not all glioblastoma cells can complete apoptosis under these conditions, suggesting the presence of additional intracellular factors that may hinder DFF40/CAD/CPAN function. By enhancing DFF40/CAD/CPAN activity, we aim to eradicate residual tumor cells and prevent recurrence. Furthermore, combining DFF40/CAD/CPAN-activating therapies with existing treatment regimens may provide a synergistic approach to improving patient outcomes. This strategy holds promise not only for glioblastoma but also for other cancers where apoptotic resistance constitutes a major therapeutic hurdle.

Conclusions

Identifying and understanding the critical *point-of-no-return* in cancer treatments is essential for preventing relapses. Currently, up to 14 distinct *Regulated Cell Death* (RCD) pathways, also known as *cell death subroutines,* have been identified.[92] Once activated, these pathways share the ultimate goal of structurally disrupt the cell's ability to maintain energy gradients with regard the exterior cellular environment, disturb the internal energy costs required to maintain these cellular gradients, or directly inducing the structural elimination of nucleus, the cellular hardware that houses the programs necessary to organize the cell as an open systems, in physic terms. However, in cancer cells, elevated entropy and the resulting loss of structural integrity (Garland 2013), may undermine the tightly regulated cellular processes required to drive the cell toward an irreversible commitment to cell death.

Defining the precise *point-of-no-return* in cell death has long eluded researchers. While process such as caspase activation or membrane permeabilization are frequently linked to irreversible cell death, their roles in determining cell fate are complex and context-dependent. In contrast, nuclear fragmentation represents a definitive and irreversible commitment to cell death, with no known cellular mechanism capable of reversing this event one initiated.

From a clinical perspective, improving patient outcomes requires how cancer treatments interact with and activate the key components of RCD to reach a true *point-of-no-return* in cell death. This knowledge is essential for interpreting the limited efficacy or failure of therapies and for elucidating underlying resistance mechanisms. Glioblastoma provides a clear example of this challenge, exhibiting deficiencies in DFF40/CAD/CPAN expression and activation. Consequently, glioblastoma cells fail to execute proper nuclear fragmentation and do not reach the *point-of-no-return* in cell death. This impaired activation not only increases the likelihood of cell survival but may

also promote genomic instability and adversely affect the inflammatory tumor microenvironment. Clinically, these deficiencies correlate with poor therapeutic responses, shorter progression-free survival, more aggressive recurrences, and potential resistance to immunotherapies. This is reflected in the limited progression-free survival rates observed in glioblastoma patients following first-line treatments and the lack of efficacy observed in clinical trials targeting relapsed disease.

Expanding therapeutic research to include strategies that enhance the capacity of cancer cells to undergo irreversible death offers a promising avenue. Advances in this area could provide valuable insights to refining current treatments and potentially lead to novel therapeutic agents with synergistic effects, thereby improving the overall efficacy of cancer therapies.

Keywords

cell death | DFF40/CAD | glioblastoma | point-of-no-return | therapeutic resistance

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Authors Contributions

M.A. prepared the original draft of the manuscript. M.A., J.B., and V.J.Y. reviewed, edited, and made revisions of the manuscript. All authors approved the final manuscript.

Affiliations

Neuro-Oncology Unit, Hospital Universitari de Bellvitge-ICO L'Hospitalet (IDIBELL), Barcelona, Spain (M.A., J.B.); Cell Death, Senescence and Survival Group, Departament de Bioquímica i Biologia Molecular and Institut de Neurociències, Facultat de Medicina, Universitat Autònoma de Barcelona, Campus de Bellaterra, Bellaterra, Spain (M.A., V.J.Y.)

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