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Cell Death in Genome Evolution

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Abstract

Inappropriate survival of abnormal cells underlies tumorigenesis. Most discoveries about programmed cell death have come from studying model organisms. Revisiting the experimental contexts that inspired these discoveries helps explain confounding biases that inevitably accompany such discoveries. Amending early biases has added a newcomer to the collection of cell death models. Analysis of gene-dependent death in yeast revealed the surprising influence of single gene mutations on subsequent eukaryotic genome evolution. Similar events may influence the selection for mutations during early tumorigenesis. The possibility that an early random mutation might drive the selection for a cancer driver mutation is conceivable but difficult to demonstrate. This was tested in yeast, revealing that mutation of almost any gene appears to specify the selection for a new second mutation. Some human tumors contain pairs of mutant genes homologous to co-occurring mutant genes in yeast. Here we consider how yeast again provide novel insights into tumorigenesis.

Keywords

Programmed cell death; apoptosis; tumorigenesis; evolution; cancer progression; yeast

1. Introduction

The evolution of genetic mutations leading to human tumors has been unequivocally verified by genome sequencing. Cancer mutations appear to originate predominantly from random replication errors, but why rare mutations are selected to become dominant cancer genotypes is much less clear. First supported by decades of studies on microorganisms grown under different conditions, human cells with a rare tumorigenic mutation are presumed to increase in number if such mutation provides a growth or survival advantage

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over other cells (e.g. evading cell death) in a particular microenvironment. This is supported by landmark studies showing that targeted chemotherapies drive the selection for drug-resistant patient tumors with mutations in the precise target site of the drug [1]. However, the exact nature of the selection pressures in tumor microenvironments (other than drugs), and the importance of specific preexisting mutations in driving the selection process are largely unknown and challenging to decipher. This gap in knowledge likely contributes to the major hurdles in translating laboratory findings into successful clinical treatments.

The use of microorganisms to further understand cancer evolution has been thwarted in part by the disproportionate downturn in funding and by biases regarding the applicability of microbial processes to human disease. Given the experimental circumstances under which programmed cell death was discovered in developing animals, it is understandable why programmed cell death was originally assumed to arise during evolution with multi-cellular organisms. As discussed in this review, these ideas have given way to the model that programmed cell death evolved earlier with the origin of cells. However, cell death pathways in microorganisms are largely unknown, and in some cases still doubted, but are also fertile for exploitation. How many genes, when activated by stress conditions, might be capable of promoting cell death? New strategies to test every non-essential gene of *Saccharomyces cerevisiae* for the ability to promote cell death revealed surprising answers. Several hundred different genes, when deleted individually, greatly increase cell survival [2]. However, death-resistance for many of these knockout strains may be due to acquired secondary mutations. Further analysis in yeast provides compelling evidence that a preexisting mutation (the yeast knockout gene) is sufficient to drive the selection for specific cancer-like mutations (inappropriate growth and/or death phenotypes) [3]. That is, the second mutation is specified by the first mutation, and new meaningful mutations are common occurrences (found in at least half of all knockout strains) [3]. This is logical, the simple selection for spontaneous suppressor mutations, but the ramifications are far from mainstream thinking in mammalian biology, tumorigenesis, and other disease states. These studies have led to new insights about cell death in genome evolution, offering new perspectives on cancer progression.

2. Evolving concepts of cell death from an historical perspective

Scientific paradigm shifts are critical to advancement of knowledge, but also present biases that are difficult to overcome. To appreciate the challenge of understanding how programmed cell death (PCD) arose during evolution and how it may impact human disease, it is useful to consider how our current understanding of PCD arose. In the mid-19th century, scientists recorded their observations of naturally occurring cell death during metamorphosis of insects and amphibians [4]. Decades later, scientists discovered that cells commonly die in many developing tissues in animals [5]. These observations lead to the assumption that such occurrences of physiological cell death during development were passive and inevitable – like a car that runs out of gas [5]. Key experimental evidence from Victor Hamburger and Rita Levi-Montalcini revealed that during development of the nervous system, many newborn neural cells die shortly afterwards due to the absence of growth factors secreted from supporting tissues [6]. In their model system, nerve growth factor (NGF) suppressed the death of developing neurons [7]. The presumed deliberate deletion of

cells when there is a limited supply of extracellular survival signals became recognized as a general strategy to control cell numbers in animals [8]. It was still another major conceptual shift to appreciate that the dying cell itself contributes to naturally occurring cell death. The idea of cell suicide was supported by early evidence that cell death could be suppressed by inhibiting transcription or translation in dying cells, which helped to stimulate the search for the macromolecules responsible for cell suicide [9, 10].

With growing improvements in microscopy technologies, scientists started to observe different morphologies of dying cells. In 1972, the term “apoptosis” was applied to describe cells traversing a series of definable morphological changes during cell death [11]. The term apoptosis was also adopted to distinguish the concept of deliberate cell suicide from the more general dead cell descriptor “necrosis”. Thus, apoptosis became synonymous with programmed cell death, as suggested by the authors [11]. The term apoptosis still retains this functional definition for many who study cell death mechanisms of yeast and other single cell species [12, 13]. However, the evidence that apoptosis indeed occurs by PCD in the early rat studies [11] was limited to morphological analysis (rather than genetic or biochemical). Therefore, most reserve the term apoptosis for the morphologically distinct cell death ascribed to both natural and experimental conditions observed during the original rat studies [11]. It took another two decades to begin to understand the molecular mechanisms of apoptosis. Using the genetic model organism *C. elegans*, a series of genes were identified as the regulators and mediators of the first defined cell death pathway starting with the decision to die followed by dismantling of the cell and ending with engulfment and degradation of the dying cell [14, 15]. Importantly, these key factors including CED-3/caspase and CED-9/Bcl-2 are conserved in mammals, providing valuable insights into the mammalian apoptosis pathway [16, 17]. Over time, the term apoptosis has evolved to a more restricted biochemical definition of caspase-3/-7-mediated cell death, as these caspases appear to be primarily responsible for the classical morphological characteristics of apoptotic cell death. This new scientific field of study flourished with intensity, yet it was assumed that apoptosis was the only programmed pathway for deliberate cell suicide. However, subsequent studies primarily in mammalian systems have produced over a dozen new terms to describe different cell death mechanisms [18-20] (see section 3.2). Developing animals were the best models for discovering evolutionarily selected apoptosis mechanisms, thus it is logical that the discovery of gene-dependent cell death in single cell species was delayed, but is now primed for new discoveries.

3. Clarification of terms in the cell death field

3.1. Programmed versus gene-dependent cell death

Before addressing the fundamental question of how PCD arose during evolution, we first clarify the terms used to describe cell death and recent controversies (Figure 1). “Programmed cell death” was originally applied by Richard Lockshin to describe cell death that occurs in a predictable time and place during the developmental plan of an organism (e.g. embryogenesis) [21]. Recently, the Nomenclature Committee on Cell Death recommended that PCD be extended to other physiological, presumably evolutionarily selected mechanisms, such as the death that occurs during adult tissue homeostasis [22].

However, in the current literature, PCD is generally applied even more broadly to include all gene-dependent cell death, including models of disease progression, necroptosis, *in vitro* models of cancer and drug therapy [23, 24], regardless of whether the death mechanism was selected during evolution or occurs accidentally (Figure 1). This is justifiable as developmental cell death genes have been shown to also cause artificial (e.g. drug-induced) cell death. Confusingly, the Nomenclature Committee refers to “accidental” cell death as uncontrollable cell death by assault [22], which may be more clearly expressed as “gene-independent” death, in which no gene products encoded by the dying cell contribute to its death (Figure 1). Furthermore, it is conceivable that gene products of the dying cell contribute unintentionally to its own demise (e.g. accidental activation of a destructive nuclease or protease that did not evolve for the purpose of promoting cell autonomous death, analogous to a centrifuge rotor that fails during a high-speed run). Underlying these nomenclature difficulties is our inability to clearly distinguish evolved versus accidental cell death processes, except in developmental processes. It is difficult to prove that cell death resulting from ischemic events was selected during evolution (e.g. to limit life span). In this case, perhaps all gene-dependent cell autonomous death was in fact evolutionarily selected. The Nomenclature Committee has also adopted more practical cell death definitions, suggesting the new term “regulated cell death (RCD)” to describe gene-dependent cell death, defined as cell death that can be modulated by pharmaceutical or genetic interventions in the dying cell [22], although this has also stirred criticism from evolutionary biologists [25]. In this scheme, PCD is a subset of cell autonomous gene-dependent RCD. Here we apply the term PCD more broadly to refer to all gene-dependent cell death, both RCD and unintentional pro-death functions not selected during evolution, justified by the experimental limitations of distinguishing evolved from accidental death functions of gene products encoded by the dying cell (Figure 1).

3.2. Morphological versus molecular definitions of cell death

The advantage of using molecular instead of morphological definitions of cell death is that it helps distinguish gene-dependent and gene-independent death [19]. Beyond evolutionary considerations, definitions of the molecular cell death machinery are also loaded with unspoken inferences. For mammals and other metazoans encoding true aspartate-cleaving caspases, the term apoptosis is generally applied only to cell death mediated predominantly by caspases-3 and -7 and the initiator caspases that activate them, such as caspase-8 and caspase-9 [26]. Caspase-3 is directly responsible for many characteristic apoptotic morphologies, DNA laddering, including blebbing, cell shrinkage, nuclear fragmentation, and chromatin condensation, while sparing plasma membrane integrity [27]. In contrast, non-apoptotic cell death by both gene-dependent and gene-independent mechanisms share necrotic morphologies, such as cell swelling and loss of cell membrane integrity accompanied by leakage of cell contents [28]. While apoptosis suppresses inflammatory mechanisms [29, 30], release of cellular contents from necrotic cells can trigger inflammatory responses through activation of TLRs and inflammasomes that activate caspases-1 and -11 [31]. Thus, inflammasome-induced programmed necrosis also requires caspases. Because cell-killing was the first discovered function of caspase-3 and other pro-death factors, the day-jobs of these factors were initially overlooked (see section 5.1). However, mounting evidence has instilled more general acceptance that pro-death/pro-

apoptotic factors have alternative day-job functions in healthy cells prior to a death signal. A new caspase biosensor reveals the widespread distribution of healthy cells that have survived caspase activity throughout most tissues of normal adult *Drosophila* [32].

The discovery that TNF α can induce necrotic cell death that is inhibited by a small molecule inhibitor of RIPK1 kinase, necrostatin-1, challenged long-held assumptions that all necrosis-like cell death was passive and uncontrolled [33]. This seminal finding provided solid evidence that necrosis can be gene-dependent and therefore druggable. Further studies identified RIPK3 as the downstream mediator of necroptosis [34-36]. Other molecularly defined cell death mechanisms include pyroptosis (caspase-1/-11-dependent) [37], keratinization (caspase-14-dependent) [38, 39] and in some cases autophagic cell death (Atg gene-dependent) [20, 40]. Many other death mechanisms have been named, including entosis [41], anoikis [42, 43], netosis [44], parthanatos [45] and many more for which the molecular mechanisms are still being uncovered. There is strong rationale to consider the possibility that there exist multiple additional pathways leading to cell death. This is consistent with how PCD may have evolved (see section 5.2).

4. When did PCD originate?

4.1. Did PCD originate with multicellular organisms or earlier?

The first breakthroughs in delineating a molecular PCD pathway came from the pioneer genetic studies in *C. elegans*. By screening mutants with abnormal cell death phenotypes, a series of genes that regulate cell death during *C. elegans* development were identified, including *CED-3*, *CED-4*, *CED-9*, and *EGL-1* [15, 46-48]. These gene products comprise the core machinery of apoptosis, which were found to be conserved in mammals, though important details differ between worms and mammals. *CED-3* encodes a protease belonging to the mammalian caspase family, which cleaves mostly unknown downstream factors to induce cell demise [16, 49]. *CED-4* encodes an adaptor protein with domains homologous to mammalian Apaf-1 (apoptotic protease-activating factor-1), which is required for caspase-9/*CED-3* activation [50, 51]. *CED-9* and *EGL-1* encode proteins of the extended mammalian Bcl-2 family [52]. *CED-9* inhibits developmental cell death and *EGL-1* is a pro-apoptotic inhibitor of *CED-9* analogous to mammalian BH3-only proteins [17, 46]. Beside the animal kingdom, PCD has also been found in plants, suggesting a crucial role for all multicellular organisms [53, 54] (Figure 2).

Multicellular organisms benefit from PCD to eliminate unwanted or damaged cells during development, homeostasis and defense against infections, which promote survival at the organismal level [55]. So, PCD may have originated from the earliest multicellular organisms as originally assumed given the animal instinct of “fight for live”. However, more recent studies have provided evidence for the existence of PCD in unicellular species, including slime molds [56], kinetoplastids [57], ciliates [58], dinoflagellates [59], fungi [60], and even bacteria [61, 62]. Thus, PCD may have originated from the last universal ancestor (Figure 2).

4.2. Why did unicellular organisms evolve PCD?

Unicellular species are not necessarily solitary cells. Yeast have been described as free-living single-cell organisms in textbooks for decades. However, this conventional definition has been recently challenged in several ways. First, instead of being single-celled forms in shaking flasks under laboratory conditions, yeast usually form colonies on solid surfaces in the environment [63]. Even in liquid media, yeast cells form clumps (also called “flocs”) through a cell surface adhesive protein encoded by *FLO1*, a gene commonly lost in most lab strains [64]. Thus, yeast are more like colonial organisms. Second, yeast harbor several types of cell-to-cell signaling. For example, haploid yeast cells tend to form diploids in the presence of their opposite mating type, as a consequence of responding to the mating-type pheromones; yeast diploids undergo efficient sporulation in acetate by converting acetate to bicarbonate as a signal to tell other cells to sporulate [65, 66]; quorum sensing mechanisms through fusel alcohols has also been identified in different types of yeast to modulate their growth and morphogenesis based on cell density [67]. If we accept that yeast are communal, the existence of PCD is more palatable.

Mounting evidence suggests that yeast cells undergo PCD in adverse environments to ensure survival of the species, rather than the individual cell. Death of aged cells has been reported to be a species-preservation mechanism in yeast colonies on solid media [68]. Aged cells enriched in the central zone of yeast colonies undergo gene-dependent cell death, resulting in the release of nutrients for consumption by younger cells localized in peripheral zones. Physical removal of old cells reduces colony survival [68]. Yeast cells were also reported to undergo PCD to ensure evolutionary advantages through mating [69]. During sporulation/meiosis, diploid yeast undergo chromosome recombination to generate genetic diversity among offspring, thereby avoiding genetic bottlenecks. PCD of some progeny spores has been observed and is suggested to ensure that sufficient biosynthetic materials are available for the remaining spores [70]. Moreover, PCD is used as a weapon for yeast to compete with other tribes. In nature, yeast strains (referred as killer yeast) are persistently infected by double-stranded RNA viruses that encode killer toxins. These killer viruses are typically lost in laboratory settings. Although communal yeast cells harboring the killer virus are immune to their own cytotoxic viral toxins, they secrete these toxins to trigger PCD in yeast cells from other communities lacking the same killer virus. This virus-host relationship in yeast is analogous to pathogenic human viruses such as herpesviruses and retroviruses that reside in their hosts but are pathogenic to new uninfected hosts. Such semblances suggest an evolutionarily conserved role of PCD in response to virus infections [71]. Thus, for unicellular organisms, the death of individual cells can benefit the long-term survival of their species.

5. How did PCD evolve?

5.1. Death — the inevitable consequence of life

To provide a general explanation for the origin and evolution of cell death genes, we consider the evolutionary contexts. What is the best strategy for a species to survive for millions of years, through unavoidable adverse environmental conditions? To increase the chances of long-term survival, genetic variation between individuals is one of the key

elements. Variation within the population of a species, together with the presence of natural selection, drives the evolutionary process. Such variation not only exists at the level of individual organisms, but also at the level of individual cells, to ensure that the fittest organisms/cells survive in a given environment. During such selection processes, PCD is an efficient way to rid of unfit cells and to reutilize nutrients for the fittest cells. But how can those death genes be selected and propagated if the cells carrying such genes all die? One explanation is that these death genes possess some pro-life function that also benefits cells and improves survival of the whole population/organism [72]. This same gene in the face of a death stimulus (e.g. adverse environmental conditions) undergoes a transition to activate its pro-death function. Possessing two (or more) functions in one gene is also an efficient strategy to achieve maximal pleiotropy with limited genetic material. A testable prediction for this hypothesis is that all the death genes should have some vital function such as maintaining a balanced metabolic state, regulating the cell cycle, etc. For example, cytochrome *c* transfers electrons between complex III and IV in the mitochondrial respiratory chain, but when released from mitochondria, cytochrome *c* activates the mammalian Apaf-1 apoptosome, an oligomeric structure that activates caspase-9 to kill cells [73, 74]. Although some have challenged the role of cytochrome *c* in cell death, the general principle appears to apply. Analogous dual functions potentially apply to all regulators of cell death, including the core apoptosis factors in mammals [75] (Table 1).

Although apoptosis-like molecular mechanisms have been suggested to exist in single cell species, alternative death mechanisms may prevail. Cell death screens of the entire ~5,000 unique knockout strains in the yeast knockout collection revealed over 800 candidate genes, when deleted, result in two-fold or better survival following exposure to a heat ramp stimulus, or to acetate concentrations compared to the wild type strains [2, 76]. Based on 1-2 month evolution experiments, cell death mechanisms are required for long-term survival of single-cell species, for example, to reduce the population to a size that is sustainable on the available resources [77]. Thus, a second explanation for how death genes evolved into existence is that in the absence of a death stimulus, the pro-death function of otherwise pro-survival genes is inhibited. However, depending on the specific context where a particular gene is being studied, the experimenter may only observe the survival or the death function, but not both. Potentially the fittest cells will survive by inhibiting pro-death gene functions through intracellular signaling. This is supported by evidence that pro-death factors, including the *Drosophila* caspase Dredd, contribute to the death of unfit cells during cell-cell competition in developing tissues [78].

5.2. Many ways to die

Considering the evolutionary perspective discussed above, variation within the population of a species ensures that there are always fit organisms/cells in a given environment. If there are many pre-existing genetic variants in a population such that a small minority of the population will contain beneficial mutations and successfully adapt to any given environment, there remains a need to eliminate unfit cells and therefore it is conceivable that there are as many ways for cells to die as there are environmental conditions. So far, relatively few types of PCD have been identified, and each usually occurs in response to a subset of signals and environmental conditions [79]. As a result, there may be many yet

unidentified PCD pathways. Our lab developed a reliable assay to detect gene-dependent cell death in yeast *Saccharomyces cerevisiae* to determine the prevalence of yeast genes that can alter cell death, regardless of their evolutionary roots. This assay is based on the viability of yeast knockout strains after treatment with precisely regulated heat ramp stimuli (a condition mild enough to allow time for gene-dependent cell death to occur but prior to adaptation), regardless of the cell morphologies during the dying process [80]. By applying this assay to the yeast knockout collection, we found unexpectedly that many gene knockout strains (~40%) are more resistant to cell death compared to the parental wild type strain [2]. This suggested that many genes can contribute to cell suicide when the environmental temperature is increased gradually [2]. We also compared different metabolic states when death was induced by heat treatment versus acidic acid, the primary organic acid that accumulates in overgrown cultures of yeast. The results often differ between different gene knockout strains [2]. These findings are consistent with the existence of many cell death pathways.

6. PCD in cancer progression — evolutionary insights from yeast

Human disease is typically accompanied by too little or too much cell death [81, 82], cancer being the hallmark [83]. Like natural selection, cancer progression is an evolutionary process selecting for the fittest cells in a particular niche [84-86]. However, because almost all random mutations are detrimental, cancer mutations also decrease overall fitness while temporarily increasing fitness in a specific niche, followed by cell proliferation that further increases genetic diversity through basal DNA replication errors [87]. It is challenging to estimate the basal mutation rate as current technologies cannot reliably identify a single point mutation difference between two recently divided sister cells [88]. While this vastly underestimates raw mutation rates, it facilitates the identification of meaningful mutations that have become prevalent in the population. The mechanisms by which any random mutation becomes prevalent in a population has been studied extensively, including estimates of benefit ratios and have been applied to understand tumorigenesis [89]. Drug-driven selection has provided important insights into selection pressure [90]. Given the genetic diversity even among closely juxtaposed cells in tumors and normal tissues, it is conceivable that prescribed death pathways must initiate at countless different points. If there are indeed many different ways for cells to die, then perhaps all cancer cells can be killed with the right stimulus if we had sufficient knowledge of the nature of PCD evolution to treat this malicious disease.

To provide experimental evidence in support of the hypothesis that almost any functional mutation is sufficient to drive the selection for compensatory mutations, the yeast knockout collection was used as a model. Unlike cancer cells, the first mutation is known – the engineered knockout gene itself [3]. The majority of yeast knockout strains have accumulated random genetic drift of unknown consequences (passenger mutations). Strikingly, an estimated 75% of knockout strains have a single meaningful point mutation not present in parental strains or other knockout strains that confers strong cell death and/or growth control (nutrient-sensing) defects (Figure 3A). Importantly, these phenotypes were not detectable under normal/rich growth conditions, but only when knockout cells were briefly heat-stressed or grown with partially depleted nutrients [3]. These meaningful

mutations however, do not appear to be fortuitous, but rather to be selected specifically as a consequence of the first mutation.

Analogous to human cancer cells, most investigators have long suspected that knockout collections of yeast and other species contain secondary mutations. However, the major reason for acquiring these secondary mutations was surprising, and inspires an alternative viewpoint regarding cancer. Random selection and environmental conditions are commonly blamed for the occasional problems experienced while using strains from the yeast knockout collections, including the stresses incurred in the laboratory during the mechanics of knockout construction [91, 92]. Indeed these extracellular environmental conditions are contributory, and even required, but genetic manipulation apparently contributes minimally [3]. Instead, an important driver for the selection of these secondary mutations in knockout strains of yeast is the consequence of losing the function of each unique original knockout gene. This is supported by the observation that independently constructed knockouts of the same gene are likely to evolve a secondary mutation causing the same secondary phenotype [3]. Remarkably, a significant portion of the paired independent knockouts had acquired secondary mutations in the same gene/protein complex based on genetic complementation tests (Figure 3A). Importantly, genome sequencing demonstrated that the secondary mutations were all unique nucleotide changes even when they occurred in the same secondary mutant gene, testifying that these are *de novo* mutations and were not inherited from parental strains [3]. Therefore, deletion of almost any single gene in a eukaryotic genome appears likely to have a major, currently underappreciated role in driving the selection for a secondary compensatory mutation. Although the yeast genome is much smaller than the human genome and may have a higher basal mutation rate (e.g. in haploid strains), the frequency of new gene mutations in the human genome approximates that of yeast when the number of cell divisions required to generate an adult (several trillion) along with the estimated rate of cell replacement (many billions per day) are taken into account [3].

By performing complementation experiments and genome sequencing, we found that knockouts of different genes sometimes can evolve mutations in the same complementation group or even in the same secondary gene (Figure 3A). When different knockouts evolve mutations in the same secondary gene, those are usually unique mutations, demonstrating independent evolution. More interestingly, in some cases, knockouts of different components of the same protein complex (e.g. subunits of Elongator complex and subunits of EGO complex) have evolved similarly. This finding further supports the idea that the loss-of-function mutation of the original gene is a key driving force to select for specific secondary mutations. Such convergent evolution observed in yeast suggests that mutations in different genes can select for common driver mutations in cancers, which also provides a potential explanation for the lack of shared mutations between tumors.

Because human tumors have a very large number of sequence differences, even between different cells in the same tumor, it may be impossible to decipher which mutation was the first to drive cancer, or whether a driver mutation in fact applies any selection pressure to evolve a secondary mutation [93, 94]. For example, few investigators seriously consider that *TP53* mutations either drove the selection for specific mutations or might compensate for

other specific genetic deficiencies. In contrast to cancer genomes, the primary mutation/deletion and the evolved secondary mutation acquired by yeast knockout strains can be identified with relative ease by sequencing two sister/cousin strains sharing the same abnormal phenotype inherited from a common ancestor. We queried the human cancer genome for pairs of mutant genes corresponding to the two co-occurring mutant genes in yeast (the engineered knockout gene and the acquired secondary mutant gene), and found many mutant gene pairs co-occurring in the same tumor at frequencies significantly greater than expected given the overall frequency of mutations in these individual genes [3]. The potential relevance of these paired cancer mutations is intriguing but yet unexplored.

These findings in yeast are consistent with cancer models in which human cells accumulate random mutations during cell division, some of which may select for cancer driver mutations and eventually lead to tumorigenesis (Figure 3B). Cell death studies in yeast may provide additional insights into tumorigenesis. Approximately 15% of the non-essential genes in the yeast genome, when deleted, resulted in acquisition of a secondary mutation that caused these cells to overgrow in low amino acid conditions compared to wild type, although they grew indistinguishably from wild type on rich medium [3]. This ability to ignore signals that normally limit cell growth is a phenotype shared by many cancers. Paradoxically, yeast strains harboring these overgrowth mutations often die more readily when stressed compared to their siblings (Figure 3A). This evidence emphasizes that evolved cancer mutations decrease (not increase) overall fitness, and are likely to provide growth and/or survival advantages at a fitness cost under other conditions. These observations instill optimism in the search for cancer therapies.

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Figure 1. Definitions of different types of cell death
PCD (programmed cell death), RCD (regulated cell death), see text.

Phylogenetic tree of pro-death factors

Figure 2. Programmed cell death appears to exist in all living kingdoms
Reported death factors are listed for organisms from each kingdom. *see Refs [2, 3].

Figure 3. Models of genome evolution in yeast and cancer cells

A. Independently constructed knockouts lacking the same gene tend to undergo parallel evolution by acquiring secondary mutations in the same genes or protein complexes, indicating a selection process driven by the knockout. The majority of the yeast knockout strains have genetic heterogeneity caused by random replication errors that are selected in the population. Deletion () of different genes sometimes result in convergent evolution, indicating these gene knockouts may apply similar selection pressures that drive the selection for common secondary mutations. Yeast cells harboring secondary mutations (stars) can grow more robustly, but only under certain adverse environment (e.g. limited nutrients), and are often more vulnerable for cell death under other conditions such as heat stress or drug treatment.

B. Hypothesized cancer genome evolution model. Random mutations generated in human cells by normal DNA replication errors can apply selection pressure for further mutations, some of which may be cancer driver mutations that are responsible for tumorigenesis. Mutations in different genes can select for common cancer driver mutations.

Table 1
Prominent examples of dual functions of cell death factors

Protein	Role in PCD	Vital function
Caspase-8	Initiator caspase promotes apoptosis; suppresses RIPK1/3-mediated necroptosis [95]	Promotes cell proliferation and differentiation [96, 97]
Bcl-xL	Anti-apoptotic full-length protein promotes neuronal cell when cleaved following ischemic injury [98-100]	Regulates mitochondrial dynamics and energetics [101-104]
Cytochrome <i>c</i>	Co-factor for apoptosome assembly and activation of caspase-9 [61]	Essential component of the mitochondrial respiratory chain [62]
Bad	Inhibits Bcl-xL to promote cell death [79, 105]	Regulates metabolism and gluconeogenesis [106, 107]