Seminars in Cell & Developmental Biology 39 (2015) 3–11

Contents lists available at ScienceDirect

Seminars in Cell & Developmental Biology

journal homepage: www.elsevier.com/locate/semcdb

Review

# Cell death in genome evolution

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ARTICLE INFO

*Article history:*
Available online 25 February 2015

*Keywords:*
Programmed cell death
Apoptosis
Tumorigenesis
Evolution
Cancer progression
Yeast

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 any 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.



**Contents**



## 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 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

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http://dx.doi.org/10.1016/j.semcdb.2015.02.014
1084-9521/

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 *Caenorhabditis 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 (Fig. 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 (Fig. 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 (Fig. 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