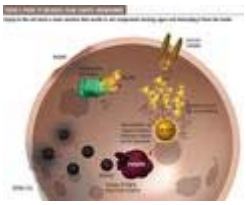
Death by misadventure

Click to Print

15 February 2003
From New Scientist Print Edition. Subscribe and get 4 free issues.
Nektarios Tavernarakis


Enlarge image

FRANÇOIS JACOB, one of the pioneers of molecular biology, once said "the dream of every cell is to become two cells". It is the cell's worst nightmare to die unexpectedly. Unfortunately, accidents happen. Cells get injured and succumb to necrosis, from the Greek for "dead", necros, a word that carries overtones of dismay.

Necrosis was first described about 15 years ago and has enjoyed something of a dismal reputation ever since. It has been a poor relation to another, more exciting type of cell death called "apoptosis", or programmed cell death. Apoptosis is active, organised and creative; necrosis is little more than disorganised rotting. Or so cell biologists thought. Now, though, the tools of modern molecular biology have given necrosis a new image. Underneath the chaos there appears to be an orderly sequence of events that recurs in most necrotic cells, and this discovery is giving us hope of finding new therapies for many debilitating diseases as well as making us question one of the fundamental concepts in cell biology.

When cell death first captured biologists' attention in the late 1980s, it was perhaps inevitable that apoptosis would take centre stage. Until then no one had realised that almost all multicellular organisms have cells that are born to die. These cells' demise is genetically programmed and when their time comes, death is orchestrated by the regulated expression of dozens of genes. Apoptotic cells are taken out through a series of paced, orderly events that result in the cell being gradually dismantled and assimilated into surrounding cells and tissue.

Apoptosis quickly became highly fashionable, culminating in the 2002 Nobel prize for Sydney Brenner, Bob Horvitz and John Sulston for their work on programmed cell death and development in a simple nematode worm, Caenorhabditis elegans.

These pioneering studies revealed the molecular players in programmed cell death and paved the way for the identification of similar genes in higher organisms, including humans. We now have a detailed understanding of the process. Far from being detrimental to an organism, cell death is a vital and creative process that is essential for development, shaping organs and tissues such as the nervous system. If programmed cell death fails we run into all sorts of trouble, ranging from developmental defects to cancer.

On the surface at least, necrosis could not be more different. It appears to be unplanned, destructive and chaotic. Unlike programmed cell death, no biochemical processes have evolved specifically to carry it out. Cells suffer necrosis when their defences are overwhelmed by unexpectedly severe conditions such as lack of oxygen or nutrients, high temperature, toxic or corrosive chemicals, or physical injury. Mutations can also trigger necrosis and this is one of the leading causes of several neuro-degenerative diseases including Alzheimer's, Huntington's, Parkinson's and amyotrophic lateral sclerosis, a form of motor neuron disease also known as Lou Gehrig's disease.

Despite the central role of necrosis in many devastating human conditions, we understand surprisingly little about it. Compared with programmed cell death, scientific progress on necrosis has been painfully slow, partly because the dominant idea has been that necrosis is a passive, disorderly process. Such a mindset does not inspire in-depth investigation. We have also lacked good models of necrosis in simple experimental organisms such as C. elegans or the fruit fly Drosophila melanogaster.

But now both obstacles have largely gone. Recent observations in a number of different animals, including primates, are beginning to overturn the grim picture of necrosis. It seems there is method behind the madness both in the overall appearance and internal structure of necrotic cells. What is more, the various injuries that end in necrosis trigger similar patterns of cellular destruction. Such observations suggest an underlying orderliness that may represent a core "necrosis program" that is activated upon injury and ravages the cell.

This realisation has given the green light to investigations aiming to dissect necrotic cell death at the molecular level. Thanks to many researchers, including my own team at the Institute of Molecular Biology and Biotechnology in Heraklion, Crete, we now have working models of necrosis in both C. elegans and Drosophila.

With the help of the animals' genome sequences, we have worked out which genes are required to carry out necrosis and what they encode. Several conditions inflict necrotic death on specific neurons in C. elegans. Afflicted worms behave abnormally - for example, they may be paralysed or uncoordinated in their movements. Tens of thousands of these worms can be exposed to mutation-causing chemicals or radiation. If a gene required for necrosis is wiped out in the worms' offspring then the neurons carrying that mutation will not die and the animal will stand out of the crowd because it will be able to move normally. That makes it relatively easy to isolate the gene and work out its cellular role.

Intriguing details of the necrotic process are now starting to emerge. Unlike apoptosis, there are no specific genes or biochemicals dedicated to carrying it out. Instead, normally innocuous cellular processes turn rogue and attack the cell. One culprit appears to trigger necrosis above all others: destructive enzymes leaking from bag-like structures inside the cell called lysosomes.

Lysosomes are intracellular "recycling centres" whose job it is to engulf and break down defunct cellular machinery, or material absorbed into the cell from outside. They contain over 80 types of enzymes, including a class of general-purpose and highly destructive protein-digesting enzymes called cathepsins. Recent investigations by several research groups, working with organisms as diverse as worms and monkeys, implicate cathepsins flooding the cytoplasm as a major cause of necrotic cell death. For example, Tetsumori Yamashima's team at Kanazawa University in Japan have studied necrotic injuries in the hearts and brains of rats and monkeys. They found that leakage most likely occurs because the lysosome's membrane is ruptured. Once these enzymes are out, they wreak havoc, trashing the cell and interfering with metabolism. At this point death is imminent. In essence, lysosomal enzymes are the cell's executioners.

But what causes these deadly enzymes to spill out of lysosomes? Several lines of research in different organisms incriminate the same suspect: calcium. Many necrosis-initiating stimuli seem to raise the concentration of calcium ions inside the cell, setting off an inexorable cascade that ends in death.

The concentration of calcium inside the cell can increase via two routes. Calcium can flood in from outside through dedicated channels on the cell's outer membrane. Or the cell can release its own calcium stores, which are mostly kept inside a network of sacs and tubules called the endoplasmic reticulum (see Diagram). Either or both of these mechanisms come into play when necrosis begins. For example, during a stroke, specialised ion channels on neurons called the NMDA receptors get jammed open and allow a cataclysmic flood of calcium into nerve cells. And while no one knows why the endoplasmic reticulum lets go of its calcium, it clearly plays a part in necrosis, because blocking the release using chemicals or mutations can sometimes stop death.

The next step in the cascade is the activation of a group of enzymes called calpains. Their normal job remains somewhat obscure - they are switched on by calcium ions and seem to break down proteins that organise the cell's internal scaffolding. But when they encounter massive bursts of calcium they become hyperactive and turn rogue, attacking the lysosome's membrane and rupturing it. Cell death quickly follows.

The evidence for this model of necrosis is now quite good. Genes for proteins involved in calcium regulation, as well as genes for lysosome enzymes and calpains, have turned up in searches for genes that stop nerves degenerating in C. elegans. And something similar seems to happen in cultured mammalian neurons.

More important hints into the mechanism of necrosis have come from studies of heat-shock proteins. These are molecular "chaperones" that help other proteins to fold properly or repair them after damage. Elegant studies with Drosophila done by Seymour Benzer's group at Caltech and Nancy Bonini's group at the University of Pennsylvania in Philadelphia have shown that elevated activity of several heat-shock proteins protects cells against necrosis caused by clumps of mutant protein. These clumps are typical of neurodegenerative diseases such as Huntington's and are thought to trigger necrosis by somehow clogging up the cell. The involvement of the heat shock system should help us work out exactly what's going on.

Clearly more research is required before we understand all the molecular mechanisms involved in necrosis. Already, though, we have gained a surprising insight from the research. While the

fundamental distinction between apoptosis and necrosis is obvious in certain situations, in others the dividing line is blurring. Detailed molecular investigation of death mechanisms indicates that the long-standing distinction between the two kinds of cell death is an over-simplification.

For one thing, programmed cell death doesn't always happen as part of development. Rather like necrosis, it can be switched on inappropriately in response to injury. All cells carry a copy of the death program and under certain stressful conditions this program can be activated.

More intriguingly, some dying cells simultaneously show features of both programmed cell death and necrosis, prompting the intriguing idea of a third type of cell death, paraptosis. This appears to be a halfway house. It does not require all of the proteins and other factors that are involved in programmed cell death, and a paraptotic cell does resemble a necrotic one. But like programmed cell death, paraptosis requires the switching on of gene expression, a known prerequisite for programmed cell death.

It is also becoming clear that what determines how a damaged cell dies is the extent of its injury. Brutal injuries will trigger necrosis while relatively mild ones give the cell time to mobilise its apoptotic death programme. The emerging theme is that instead of two distinct types of cell death, there is a continuum of responses with two extremes, one being programmed cell death and the other necrosis.

If true, the new view of necrosis could have important practical spin-offs. Perhaps there is another way to stop necrosis without removing the initial trigger. Could we develop methods of fortifying cells against injuries that would otherwise be lethal? The question is particularly important since necrotic cell death is a key problem in human health. Apart from its role in many neurodegenerative disorders, necrosis associated with strokes, heart attacks and kidney disease is a major cause of death and disability.

Obviously there are extreme insults that will quickly inflict unavoidable death - for example, permanently severing a cell's oxygen supply, or shattering it with a bullet for that matter. But the great majority of necrotic deaths are caused by relatively mild offences. In those cases cells might be reinforced to withstand the death-inducing conditions through genetic engineering or drugs. Recent research hinting at a common necrosis program could make such a goal attainable. The molecules enacting these biochemical events can be considered global effectors of necrosis and make excellent drug targets.

One particularly exciting finding is that inhibitors of lysosomal enzymes significantly reduce necrotic cell death in C. elegans and cultured mammalian cells, as do inhibitors of calpain and agents that restrict concentrations of calcium inside cells. As well as backing the new view of necrosis, these discoveries emphasise how understanding necrosis could lead to new therapies that prevent or ameliorate necrosis in humans. Necrosis might once have been seen as a dead end, but it is now an area of research that is very much alive and kicking.

From issue 2382 of New Scientist magazine, 15 February 2003, page 30