# Pathway to cell death redefined in landmark study

September 20 2007

A new study led by investigators from the University of Pittsburgh School of Medicine demonstrates that the process of necrosis, long thought to be a chaotic, irreversible pathway to cell death, may actually be triggered as part of a regulated response to stress by a powerful protein, SRP-6, that can potentially halt necrosis in its path. Further, the research team realized that this protein might be harnessed to direct some cells -- those in cancerous tumors, for instance -- to die, while saving others, such as degenerating neural cells responsible for Alzheimer's and Parkinson's diseases. The work appears on the Sept. 21 cover of the journal *Cell*.

This remarkable molecular trigger, SRP-6, is a serine protease inhibitor or serpin, and targets the cell's digestive center, the lysosome. The authors report that the family of intracellular serpins may help cells survive in the face of stressors by protecting against lysosomal injury and its cellular consequences.

"For years, we believed that cell death related to a catastrophic insult such as a stroke or heart attack that deprives tissue of oxygen couldn't really be treated, so we focused on strategies to prevent further damage by restoring blood flow as quickly as possible with clot busters and surgery," said Gary A. Silverman, M.D., Ph.D., chief of newborn medicine in the department of pediatrics at the Pitt School of Medicine and the study's senior author. "But our research indicates that necrosis can be interrupted and possibly repaired, even after the injury process is well underway. This insight has exciting implications for the

management of heart disease, stroke and neurological illnesses."

Representing more than five years of study, the Cell publication is the result of a chance observation made by primary author Cliff J. Luke, Ph.D., assistant professor of pediatrics at Pitt and an investigator at the university-affiliated Magee-Womens Research Institute. Drs. Luke, Silverman and colleagues have been studying how a certain class of proteins called proteases, when uncontrolled, can kill cells. In the process, they discovered that another group of proteins, the serpins, might block, or inhibit, these destructive proteases and protect cells from injury. SRP-6 is among a vast family of pro-survival serpins, which are key regulatory molecules in many complex biologic processes, including blood cell coagulation, inflammation, tumor growth and cell death. Although previous research has shown that bloodstream serpins, including antithrombin and alpha-1 antitrypsin, control protein degradation, little is known about the role of serpins that function within cells, especially in a living organism.

Enter serendipity. When collecting specimens of a microscopic worm called Caenorhabditis elegans in water, rather than in a saline solution as is more common, Dr. Luke noticed that an extraordinarily large number of the animals were dying. "My worm yield was way down," he said. When he examined the dying worms, he determined that they were genetic "knock-outs" that had been modified to be deficient in SRP-6. The normal worms were just fine.

A frequently studied animal model because of its 1,000-cell structure, transparency and easily visible development, C. elegans is a primitive organism whose complete genetic code has been sequenced and is well known to scientists. The worm typically lives in soil, flourishes in water and exists to eat bacteria and reproduce. The investigators were using a "reverse genetic" approach in which they hoped, by studying the relatively limited intracellular serpin repertoire of C. elegans, they could

gain insights that might be applicable to serpin function in higher organisms, including humans.

“Serpin proteins are critical,” said Dr. Silverman, a neonatologist and a senior investigator at the Magee-Womens Research Institute. “For example, we know that in patients who have a certain type of skin cancer, those whose tumors express a lot of intracellular serpins don’t do as well. Now we know that SRP-6 is a crucial pro-survival mechanism that can protect cells from injury, initiate repair after injury, or, if absent, lead to a cascade of cell death.”

With further investigation, it may be possible to use this knowledge to deprive cancer cells of their serpin protectors and target them for death. Alternatively, physicians might be able to boost serpin activity to stop cells from dying – for example, intestinal cells affected by the bacterial infection necrotizing enterocolitis (NEC), a major cause of death and illness in fragile, premature infants.

“We still treat NEC the same way we did 30 years ago, with supportive care, antibiotics and surgery to remove dead portions of intestine,” said Dr. Silverman. “We can’t stop the mucosal lining from dying. But with these worms as models, we can do drug screens to search for compounds that can block necrosis.”

Drs. Silverman, Luke and colleagues have dramatically illustrated the devastating consequences of cellular stress in C. elegans when the crucial protector SRP-6 is missing. A cascade of cell necrosis begins in SRP-6-deficient animals exposed to a number of different stressors, including water, heat and lack of oxygen. In the case of water exposure, the SRP-6 knock-outs move a bit but soon become immobile. Finally, the worms’ organs are violently expelled through their bodily openings, resulting in what the authors refer to as a “grim fate.”

“Animals with normal genetic sequences are fine in water, but the knock-out animals usually die rapidly,” said Dr. Luke, explaining that this observation led him to realize the importance of SRP-6 in protecting the lysosome, an internal cell structure enclosed in its own protective membrane that acts as the cell’s garbage disposal. Powerful enzymes within the lysosome digest old, worn out proteins, carbohydrates, lipids, DNA, RNA, other damaged cell structures and even invading bacteria and viruses. But if the lysosome becomes damaged and leaky, these enzymes can turn against the cell and possibly overcome the serpin defense – useful if the cell is part of a cancerous tumor.

The investigators determined that SRP-6 staves off necrosis by protecting the lysosome membrane from damage caused by the calpain family of cysteine proteases and by neutralizing other cysteine proteases released from injured cellular structures called organelles as they are being digested by the lysosome. As part of their study, Drs. Silverman, Luke and colleagues labeled enzymes within the lysosomes of SRP-6-deficient animals with a fluorescent biomarker to observe how these enzymes reacted after an injury to the critical structure.

“The lysosomes popped, released their contents into the cell and these digestive enzymes began to activate, making the whole animal fluoresce,” said Dr. Silverman. “Again, this experiment showed the importance of SRP-6 in management of the necrosis pathway.”

“There are a lot of diseases associated with cell necrosis, such as stroke, neurodegenerative diseases and NEC, and now we know that the pathway to necrosis is much more systematic than we once thought it was,” said Dr. Luke. “With further study, we may be able to identify targets of intervention to halt the necrotic progression in some of these diseases and possibly even prevent them.”

Source: University of Pittsburgh