Two Faces of Evil: Cancer and Neurodegeneration

Adapted from an original article by Jennifer Altman in the September-October 2010 issue of Alzheimer Actualités, a newsletter published in French by the Ipsen Foundation. The Alzforum editors acknowledge the Foundation's generosity in making this summary freely available in English.

13 October 2010. Cancer and neurodegenerative diseases—what do they have in common, apart from the distress they cause to sufferers and their families? The answers that emerged from the discussion at this year's Fondation IPSEN Colloquium on Alzheimer's Disease lie in the complex signaling mechanisms that control the life and death of cells. The understanding of these pathways, gained mainly from cancer studies, is beginning to throw light on why neurons die prematurely in a range of neurodegenerative diseases and to open up possibilities for new therapeutics. The meeting, held in Paris on 26 April 2010, was organized by **Tom Curran**, of The Children's Hospital of Philadelphia, Pennsylvania, and **Yves Christen**, Fondation IPSEN, Paris, France.

Two key events in the lives of cells, as in the lives of our bodies, are birth and death. A further important stage in the cell's life cycle is differentiation, when it ceases to divide and commits to becoming a specific cell type with a particular set of functions. Cell division, differentiation, and cell death—also known as apoptosis—are complex processes that are tightly regulated by highly tuned networks of chemical signals which prevent too many cells being born, stop cells failing to differentiate, and protect cells from dying prematurely. Breakdowns in these signaling networks underlie both cancer, where cells divide out of control, and neurodegeneration, in which differentiated neurons die too young.

Some cells normally continue to divide throughout the life of an individual, providing a pool of stem or progenitor cells that supply tissues with replacements for cells that become worn out and die. Other cells differentiate into tissue specific cell types: The genes that regulate cell division are shut down and genes controlling the cell's particular functions are activated. As the cell reaches the end of its useful life, a further set of genes, known as the death pathway, swings into action, ensuring a safe and tidy shutdown and degradation of the cell's components. Neurons differ from cells in most other tissues because they are not replaced when they die: they are unable to revert to a dividing state, so they are known as terminally differentiated and, with a few exceptions, the progenitors disappear shortly after birth.

In both cancer and neurodegeneration, cells recapitulate their developmental process but with different consequences: A cancer cell results when mutations in crucial genes in a differentiated cell reactivate the genes that control cell division, initiating uncontrollable replication; because they do not have the option of re-entering the cell cycle, challenged neurons seem instead to enter the death pathway prematurely, explained **Karl Herrup**, Case Western Reserve University, Cleveland, Ohio (see Yang et al., 2003).

Consequently, detailed knowledge about the mechanisms of de-differentiation in cancer cells may provide a source of insight into the triggers for cell death in neurons.

Dividing and Dying

Cell division is characterized by an orderly sequence of steps—the cell cycle—which takes the cell from its resting state to the duplication of the chromosomes carrying the DNA (mitosis) and separation into two new cells (cytokinesis). Work in yeast cells established that progress from one step to the next is triggered by the interaction between two proteins, a step-specific cyclin and a cyclin-dependent kinase (Cdk). In mammals, the picture seemed to be more complex, as there was thought to be a dedicated Cdk for each step, but recent studies in mice, in which the genes coding for individual Cdks are eliminated, have now shown that this is not the case. In mammals, as in yeast, only Cdk1 is essential to drive the cell cycle, reported **Mariano Barbacid**, of the Centro Nacional Investigaciones Oncologicas, Madrid, Spain (Santamaria et al., 2007). Mammalian Cdks 2-4 and 6 are essential for cell division only in particular tissues or under certain physiological conditions. For example, Cdk4 is required for proliferation of the pancreatic β cells that produce insulin, and a few other specialized cells (Rane et al., 1999). In lung cancer, Cdk4 has a toxic interaction with the cancer-promoting gene k-ras, so inactivating Cdk4 could have therapeutic benefits (Puyol et al., 2010).

Not surprisingly, some of the Cdks have roles in the nervous system, both in the division of neuron progenitor cells (Atanasoski et al., 2008) and in mature neurons. Herrup showed that, unlike other Cdks, Cdk5 suppresses cell division, both in the developing cortex and probably throughout life, and also promotes differentiation (Cicero and Herrup, 2005). In mice lacking Cdk5, the neuronal progenitors that give rise to the cortex continue to divide rather than differentiate, many newly generated cells die, and migration of new neurons to their adult locations is disorganized. In a range of neurodegenerative conditions and in mouse models of Alzheimer disease (AD), neurons in degenerating areas contain proteins associated with cell division and some even replicated DNA, but they do not complete cell division (Yang et al., 2006; Cicero and Herrup, 2005). So far, there is no proof that these neurons will die prematurely, but their abnormal behavior is likely to compromise their function. To prevent cell division, Cdk5 has to be located in the cell nucleus and bound to a protein known as p35. This complex then binds to and inactivates E2F1, a molecule that initiates cell division (Zhang et al., 2010). Herrup concluded that mechanisms for suppressing the cell cycle thus seem to be essential for maintaining healthy tissue—their breakdown contributes to the development of cancer and of neurodegeneration.

Another molecule with protective functions is p73, a relative of the p53 protein, which is well known to cancer biologists as a tumor suppressor because it pushes unhealthy cells into the cell death pathway. In the nervous system, p73 inhibits p53, blocking apoptosis, and so contributes to the survival and maintenance of developing neurons, reported **Freda Miller**, University of Toronto, Canada. Its significance in adult animals became evident when older mice lacking one copy of the p73 gene developed signs of neurodegeneration, both behavioral and pathological, including loss of neurons and the appearance of the abnormally phosphorylated form of tau, the protein that forms

neurofibrillary tangles in AD (Lee et al., 2004). Supporting Herrup's hypothesis (above) these mice, too, have neurons re-entering the cell cycle (Wetzel et al., 2008). These changes were accelerated in a mouse model of AD that is also lacking p73. Two forms of p73 are known: a shorter version (ΔNp73) that seems to be responsible for the anti-apoptotic embryonic effects (Lee et al., 2004), and the full-length form, TAp73, which is involved in maintaining the postnatal stem cell population in the hippocampus. The ΔN form is also found in excess in several types of tumors, where it seems to antagonize the tumor-suppressor actions of TAp73 and p53.

As Frıdıric Checler, Institut de Pharmacologie Molıculaire & Cellulaire, Valbonne, France, described, several observations implicate p53 itself in AD. These include increased levels and activity of the protein in AD brains (Kitamura et al., 1997) and in vitro evidence for p53 interacting with both the amyloid-β42 (Aβ42) found in plaques and γ-secretase (Alves da Costa et al., 2006), one of the enzymes involved in releasing Aβ42 from its parent amyloid precursor protein. Presenilin, the catalytic component of γ-secretase, reduces the production of p53 from cells in vitro and in the brains of mice unable to make presenilin (Kallhoff-Munoz et al., 2008). (Interestingly, these presenilin-deficient mice also develop skin tumors.) An intricate loop of reactions regulates both p53 and γ-secretase: The production of p53 and its apoptotic action in neurons are increased by AICD, another fragment of the amyloid precursor protein generated by the secretase (Ozaki et al., 2006). In turn, p53 controls the action of presenilin on the production of PEN-2, another component of the γ-secretase complex, an action also potentiated by AICD (Dunys et al., 2009). Although not yet fully understood, this regulatory loop illustrates both the complexity and fine-tuning of the intracellular controls on which the fate of cells depends.

Neuronal Plasticity

The continual growth and replacement of new synapses as a result of experience-driven neuronal activity can be viewed as the neuron's equivalent of self-renewal by cell division. The signaling pathways within synaptic terminals, and their responses to use and disuse, are now known in some detail, but it is still unclear how defects in these pathways might contribute to some inherited conditions characterized by impaired neural development and cognitive dysfunction.

Angelman syndrome and some forms of autism have been linked to the gene coding for E3 ubiquitin ligase (Ube3A), which labels proteins destined for destruction with the small ubiquitin peptide (Kishino et al., 1997). Previously known to be essential for the cancer-initiating action of the human papillomavirus, Ube3A is also part of the mechanism regulating synaptic transmission and plasticity (Yashiro et al., 2009). The ligase determines the number of AMPA-type glutamate receptors on the synaptic membrane, according to **Michael Greenberg**, Harvard Medical School. Ube3A binds to a synaptic protein, Arc, which regulates movement of AMPA receptors in and out of synaptic terminals (Waung et al., 2008) . Both Arc and Ube3A are activated in response to synaptic stimulation, but Arc activity increases more rapidly, promoting removal of AMPA receptors from the synaptic membrane. A slower increase of Ube3A then disables Arc, allowing receptors to reappear on the synaptic membrane. In Angelman syndrome,

where Ube3A is lacking, synaptic function is impaired because too much Arc depletes the AMPA receptors in the synapse.

Arc is manufactured in the synapse in response to stimulation (Lyford et al., 1995). The control of this local synthesis depends in part on a class of small RNA molecules known as microRNAs. **Kenneth Kosik**, University of California Santa Barbara, described how the messenger RNAs for synaptic proteins, transported from the neuron's nucleus to the synapses, are maintained in an inactive state by being bound to a microRNA embedded in a complex of proteins. When a synapse is excited, a local enzyme attaches ubiquitin to the microRNA complex and packages it for degradation, freeing the mRNA for translation into protein. Once synaptic excitation ceases, the microRNA/mRNA complex reforms and translation stops. Because the volume of the synaptic space is very small, Kosik calculates that exquisite control can be achieved with very few molecules.

The surprising discovery of a molecule with properties of a functional prion-like protein affords further insight into how proteins are synthesized in excited synapses. Named cytoplasmic polyadenylation element binding protein (CPEB), this protein exists in two forms—as single soluble molecules or as aggregates, explained **Eric Kandel**, Columbia University, New York. Like the pathological prion proteins associated with prion diseases, such as Creutzfeldt–Jakob disease, aggregated CPEB perpetuates itself by recruiting soluble CPEB (Si et al., 2003; Si et al., 2010). Unlike the pathological prions, however, CPEB aggregation is stimulated by synaptic excitation and the aggregates bind to mRNAs in the synapse, triggering their translation into proteins required for synaptic maintenance. Because of its prion-like structure and behavior, keeping CPEB under control is crucial and may involve a protein modification mechanism known as SUMOylation. Targeting that process could be useful for treating prion diseases.

One feature that developing neurons share with advanced cancer cells is their ability to migrate—in the case of neurons, from their birth place to their final adult anatomical location. Migration requires the actin component of the cytoskeleton, which converts into fibrillar aggregates in response to external guidance signals. This conversion involves several helper proteins that are produced as a consequence of myocardial-related transcription factor releasing globular actin (to which it is normally bound) and moving into the nucleus. There, the transcription factor complexes with serum response factor to activate the transcription of the helper protein genes, outlined **Alfred Nordheim**, University of Tübingen, Germany. In mice lacking the serum response factor gene, neuronal migration is seriously disturbed, and cells cultured from the mice fail to adhere to culture dish surfaces and do not produce filopodia to pull them along (Knoll et al., 2006). Nordheim finds that serum response factor also seems to be required for guiding the outgrowth of axons in newborn neurons.

Building and retracting processes is one way that neurons can respond to their environment without having to undergo self-renewal by cell division. But new neurons are formed in the adult brain in the olfactory bulb and the dentate gyrus of the hippocampus. Each retains a population of stem cells, established perinatally, that give rise to neuron progenitors throughout life, according to research from the labs of Miller,

Hongjun Song, Johns Hopkins University School of Medicine, Baltimore, Maryland, and **Arturo Alvarez-Buylla**, University of California, San Francisco, among others. A range of physiological, pathological, and pharmacological stimuli promote the differentiation of progenitors and the incorporation of new neurons into the adult hippocampal circuitry, showed Song. One molecule produced by the granule cells of the dentate gyrus in response to such stimulation is Gadd45b, a protein first defined as a responder to DNA-damaging stress. In progenitors, Gadd45b supports their differentiation into neurons and the growth of dendritic trees (Ma et al., 2009).

The Gadd45b signal is not direct; rather, it activates genes that code for the neuronal growth factors BDNF and FGF, not through gene transcription, but by removing methyl groups that are attached to gene-specific regions of DNA and silence transcription. This process of methylation and demethylation is part of another level of gene regulation, known as epigenetics, which is very susceptible to external stimuli and so helps mold protein production to experience. The enormous significance of epigenetic regulation is only just beginning to be appreciated in both cancer biology and neuroscience.

Clues From the Nervous System

Of course, one of the closest overlaps between cancer biology and neuroscience is when tumors grow in the nervous system itself. Studies of developmental brain tumors led by Tom Curran, The Children's Hospital of Philadelphia, Pennsylvania, and of rare degenerative conditions conducted by **Robert Darnell**, Rockefeller University, New York, are providing new ideas about mechanisms of both cancer and neurodegeneration, as well as furthering understanding of the regulation of protein synthesis.

Childhood medulloblastoma is a cerebellar tumor that, when not fatal, leaves a lifetime of disability. Drawing on knowledge of developmental neurobiology, the origins of one subset of the tumor have been identified and a potential drug is now in Phase 1 clinical trials, reported Curran. The tumor arises through a failure of neuronal progenitor cells to differentiate because of the malfunction of a differentiation signaling pathway, the so-called Hedgehog pathway. Progenitors have a protrusion on one surface, known as a cilium, that regulates differentiation into specific neuron types by detecting the diffusible Hedgehog signal and triggering the gene transcription repressor, Gli3, explained Alvarez-Buylla (Spassky et al., 2008). Overactivation of the Hedgehog pathway, because of the lack of one component, results in the cell continuing to proliferate rather than differentiate. A Hedgehog antagonist that suppressed tumors in a mouse model of this form of medulloblastoma is showing preliminary success with patients, said Curran. Both Alvarez-Buylla and Curran considered that this also provides a proof of principle of the role of cilia and the Hedgehog pathway in the induction of cancer.

The rare degenerative diseases studied by Darnell, known as paraneoplastic neurologic diseases (for a review see Musunuru and Darnell, 2001), result from attacks on brain structures by antibodies produced by the immune system in response to proteins on tumors elsewhere in the body. Cloning DNA from patients with such conditions has led to the identification of several nervous system proteins that are sufficiently similar to the tumor proteins to come under attack by the antibodies. One such antigen is a neuron-

specific vesicle coat protein found in a patient with cerebellar degeneration (Newman et al., 1995). The most significant discovery is several neuron-specific RNA-binding proteins, such as the Nova family, discovered in a patient with failure of motor control (Buckanovich et al., 1996). The Nova RNA-binding proteins regulate the alternative splicing of mRNAs (another way of controlling the final composition of a protein), and members of this family regulate several mRNAs that encode synaptic proteins, including the inhibitory glycine and GABAA receptors (Dredge and Darnell, 2003).

High-throughput assays are detecting many more sites of interaction between proteins and RNAs, including ones involved in microRNA networks. These small RNAs, which regulate the translation of mRNAs through complex feedback loops, are involved in the control of cell identity and apoptosis, as well as local synthesis of specific proteins, explained Kosik. The discovery of both RNA-binding proteins and microRNAs is revealing the extraordinarily complex control of protein synthesis and exposing its relevance for neuronal function. It also provides another clue to failures that may result in either cancer or neurodegeneration.

Further weight to the complexity of the processes underlying disease is indicated by work on a group of devastating inherited neurodegenerative diseases that includes Huntington disease and the spinocerebellar ataxias (SCA). Each is caused by the aberrant behavior of a protein with abnormally long tracts of a single amino acid, glutamine. In SCA, the affected protein is Ataxin1, and **Harry Orr**, Institute of Human Genetics, Minneapolis, Minnesota, demonstrated that analysis of how Ataxin1 functions is revealing a two-stage process in the disease. Only the later stages are dependent on the expanded polyglutamine tract; the initiation and early progression of the disease seem to be linked to a phosphate residue attached to a single serine amino acid in a part of the protein responsible for its location in the cell nucleus (Duvick et al., 2010). Protein kinases, which attach such phosphate residues to proteins, are already being successfully targeted by anti-cancer drugs, pointing to a productive avenue for drug development in neurodegenerative diseases.

A Fruitful Pairing

Having together two groups of scientists who rarely meet—and almost didn't on this occasion because of the Icelandic volcano—highlighted just how much crossover there is between the two fields. Although the complexity of the mechanisms of cellular life and death looks daunting, the increasing understanding of these pathways offers an expanding range of therapeutic targets, as well as the possibility that drugs developed in one of the two fields will find application in the other. This cross-fertilization between the fields may well go on to bear a wonderful new crop.—Jennifer Altman.

Jennifer Altman is a science writer in Todmorden, U.K.