healing', he says, but the assay does not identify the target or even the system that is affected by a compound. Ayyappan Rajasekaran, who studies epithelial cells in cancer at the University of California, Los Angeles, CA, USA (http://www.ucla.edu/), agrees that the compound might be affecting processes other than motility. 'MDCK cells divide every 12–13hours. It is important to clarify by specific experiments that the inhibitory effect of this compound is due to inhibition of cell motility and not [of] cell growth'. Yarrow adds that with an all-or-none result, the secondary assay becomes very important to determine the target of your compound. Fenteany's group is now working to identify two candidate molecules that bind UIC-1005.

Fenteany realizes that identification of compounds using a wound assay is a far cry from therapeutic drug development. 'Even if it is very specific…what really happens when you shut down motility?' he asks. 'Those questions have to be addressed.' The best case scenario, says Fenteany, would be 'a magic bullet to bind some protein that is expressed specifically on metastatic cancer cells'. Yarrow points out that therapeutic targets will probably be upstream signaling molecules, which are more likely to be cell specific. 'Compounds that inhibit cell migration through the actin cytoskeleton might very well be more trouble than they are worth.' But there is no doubt that inhibitory compounds will be valuable research tools to determine the roles of the 100 or so proteins that associate with actin, for which there are very few specific inhibitors [3].

Exploiting cell motility

One might envisage an array of applications for compounds that affect motility, from acceleration of wound healing to cell-stopping immunosuppressant drugs. But perhaps cancer is best poised to reap the benefits of a drug that slows cell migration. In the research for anti-cancer therapeutics, says Fenteany, '99% has focused on cell growth', whereas cell motility, 'hasn't been explored as a therapeutic target'. Rajasekaran agrees that motility needs to be exploited in developing cancer treatments. 'The problem of this disease is metastasis, which involves invasion and cell motility. In my mind, therapeutic drugs that target cell motility and invasion will be as critical as drugs that restrict cell growth.'

Although the idea of exploiting cell motility in anticancer therapeutic drugs is not exactly new [4], it is one that has gained increasing momentum in recent years [5,6]. The success of such a strategy becomes more probable with each newly identified molecular player in the realm of cell motility. And understanding those players could just start with a dish of wounded kidney cells.

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News in brief

Targets and mechanisms

Leptin: missing link between obesity and breast cancer

The growth factor leptin might be the missing link between obesity and breast cancer in post-menopausal women, say scientists [1].

The findings explain, for the first time, the observation that overweight women often go on to develop breast cancer. The research also contributes to a better understanding of the disease, and will make it easier for susceptible women to be identified and treated as early as possible.

Under the leadership of Margot Cleary, a researcher at the University of Minnesota (http://www.umn.edu/), the group demonstrated that leptin, an adipocyte-derived cytokine, stimulated the proliferation of both normal and cancerous human breast epithelial cells, except that there was a pronounced difference between the two. Whereas normal breast cells proliferated by about 50%, the figure for cancerous cells was closer to 150%.

According to Cleary, 'these findings may explain why weight gain, which is accompanied by higher than expected leptin concentrations, also has been associated with increased breast cancer risk.'

Breast cancer is one of the biggest killers of women today; the World Health Organization (http://www.who.int/en/) estimates that in excess of 1.2 million people worldwide were diagnosed with

  PII: S1359-6446(02)02558-8

the disease this year. 'Preventive measures need to be taken to control these deadly diseases,' added Cleary.

1 Hu, X. *et al.* (2002) Leptin – a growth factor in normal and malignant breast cells and for normal mammary gland development. *J. Natl Cancer Inst.* 94, 1704–1711

Bilirubin outshines glutathione

Scientists have finally solved the mystery of why the body produces bilirubin, a potentially toxic molecule when found in high concentrations: it is a powerful antioxidant, which leaves glutathione, its closest rival, in the shade [2].

When Solomon Snyder and co-workers used RNA interference to silence the RNA for biliverdin reductase, the enzyme that manufactures bilirubin, they found that toxic oxidants like hydrogen peroxide accumulated and led to apoptotic cell death. But when they removed glutathione, the molecule that was once believed to be the major cellular antioxidant, they observed comparatively small increases in oxidant concentration and cell death.

The researchers, based at the Johns Hopkins University School of Medicine (http://www.jhu.edu/), now think it is bilirubin, not glutathione, that provides the cell's main line of defence against harmful oxidants. They found that a single molecule of bilirubin can deal with 10,000 oxidant molecules, whereas glutathione only reacts with oxidants on a one-to-one basis.

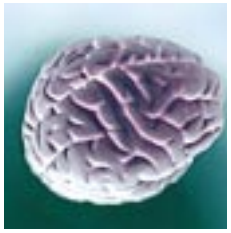
The team says they also understand how bilirubin can be so effective despite being scarce in cells. They think it is part of a cycle, which enables it to be used over and over again. 'An oxidant reacts with bilirubin to make biliverdin, which is then converted back into bilirubin by biliverdin reductase,' said Snyder.

A better understanding of the cell's antioxidant protection could be useful in the treatment of strokes and heart attacks.

2 Barañano, D.E. *et al.* (2002) Biliverdin reductase: A major physiologic cytoprotectant. *Proc. Natl. Acad. Sci. U.S.A.* 10.1073/pnas.252626999 (http://www.pnas.org)

Neurodegenerative diseases

Cholesterol drug as new MS therapy?



Researchers at the University of California, San Francisco (http://www.ucsf.edu/) and Stanford University Medical Center (http://www.med.stanford.edu/medcenter/index.html) conducted a series of studies involving mice with experimental autoimmune encephalomyelitis (EAE) to determine the impact of the cholesterol-lowering drug atorvastatin (Lipitor) on various stages of the experimental model of multiple sclerosis (MS). The researchers looked at a variety of symptoms that are related to motor control, and observed that when atorvastatin was given at the onset of the symptoms – when mice develop chronic paralysis – the drug lessened the paralysis. When the drug was given during the acute attack in the same model, it also suppressed paralysis [3].

The drug was also given to mice with relapsing-remitting disease – the most common form of MS – and the results were more dramatic. In mice experiencing their first attack, the drug prevented the MS progressing to the fully established form of the disease and in animals experiencing symptoms of their second attack the drug reversed the emerging paralysis. The findings show that atorvastatin prevented relapses or reversed paralysis in mice with the experimental form of the disease.

This suggests that statins could be useful in treating MS alone or in combination with the current therapies that are used to treat the relapsing-remitting form of the disease (Copaxone and the β-interferons) and Novatrone – a cancer chemotherapy – which is used to treat the progressive form. These drugs are often not well tolerated and are limited by side-effects; by contrast, the statins are relatively well tolerated and safe.

Scott S. Zamvil, Assistant Professor at UCSF, says that statins could also prove effective against other autoimmune diseases, such as rheumatoid arthritis and juvenile diabetes. 'The findings are encouraging in mouse studies and cell culture,' says Olaf Stuve, co-author of the study and a post-doctoral fellow in the Zamvil lab, 'The next step is to see whether there are beneficial results in people.'

3 Youssef, S. *et al.* (2002) The HMG-CoA reductase inhibitor, atorvastatin, promotes a Th2 bias and reverses paralysis in central nervous system autoimmune disease. *Nature* 420, 78–84

A new direction in neurodegenerative therapy

A promising new approach has emerged in the search to prevent the onset of diseases such as Parkinson's disease (PD) in susceptible individuals. A team led by Nancy Bonini of the University of Pennsylvania (http://www.upenn.edu/) have prevented the onset of neurodegenerative disease in the fruit fly [4].

PD is characterized by the progressive deterioration of dopaminergic neurons in certain areas of the brain. The disease has been linked to toxic levels of the protein α-synuclein. Earlier research by Bonini's group [5] had suggested that this effect could be ameliorated by the pharmacological enhancement of chaperone proteins. To test the theory, *Drosophila* were fed the naturally occurring antibiotic geldanamycin. This increases the activity of Hsp90, a molecular chaperone. Genetically susceptible flies that would normally experience a 50% loss of dopaminergic neurons after 20 days were found to have normal numbers of these neurons after treatment.

The results are exciting because they offer an entirely new approach to combating neurodegenerative disease. Traditional therapies, such as levodopa and deprenyl, only provide symptomatic relief by targeting neurons already affected by the disease. Bonini commented: 'Our studies suggest that a new class of drugs might prevent neurodegenerative disorders by fortifying these neurons even before the onset of disease.'

4 Auluck, P.K. and Bonini, N.M. (2002) Pharmacological prevention of Parkinson's disease in *Drosophila*. *Nat. Med.* 8, 1185–1186
5 Auluck, P.K. *et al.* (2002) Chaperone suppression of a-synuclein toxicity in a *Drosophila* model for Parkinson's disease. *Science* 295, 865–868

Prime time for neural repair

The dream of using stem cells to repair damage in the CNS is another step closer to becoming a reality. It now seems that the reluctance of stem cells to differentiate into neurons *in vivo* can be overcome if the cells are 'primed' before they are grafted into the CNS [6].

It is known that human and rodent stem cells can successfully differentiate into specific neurons when grafted into the developing CNS or into neurogenic areas of the adult CNS. However, such cells are reluctant to differentiate into neurons when grafted into areas of the adult brain or spinal cord that do not normally generate neurons.

Now, Ping Wu and colleagues at the University of Texas Medical Branch at Galveston (http://www.utmb.edu/) have devised a way to obtain specific neurons from stem cells grafted into both neurogenic and non-neurogenic areas of the adult rat CNS. They pre-treated foetal human neural stem cells (hNSCs) with a cocktail of growth factors and signalling molecules known to be important for development of cholinergic neurons – neurons that are required for motor function, learning and memory and that are lost in Alzheimer's disease and amyotrophic lateral sclerosis.

The team found that neurons treated with mixtures containing basic fibroblast growth factor (bFGF) and heparin could develop into cholinergic neurons *in vitro* and *in vivo* – even when grafted into non-neurogenic areas. Moreover, the resulting neurons had characteristics of the particular subtypes of cholinergic neuron normally found in each area.

Wu's group is now looking at whether the new neurons could mediate repair in rats with spinal cord injury or motor neuron disease. 'We will see if we can produce the same results in those diseased animals, and the next challenge will be to see if the neurons can actually make the right contact to the right targets', explained Wu. 'Then we'll see if they can release the neurotransmitters, and then look at function to see if there is long-term functional recovery'.

6 Wu, P. *et al.* (2002) Region-specific generation of cholinergic neurons from fetal human neural stem cells grafted in adult rat. *Nat. Neurosci.* 10.1038/nn974

Lasers lead the way for growth of neurons

Scientists have thrown out their optical tweezers in favour of laser beams, and shown that weak optical forces are enough to guide and influence the growth of neurons [7]. Their findings have opened the door to a new method of repairing damage to nerve cells without any of the damage caused by old techniques.

Allen Ehrlicher and researchers at the Department of Physics of the University of Texas (http://www.utexas.edu/) placed weak optical forces at the leading edge of neurons, called the growth cone, and in doing so, found that they could accelerate growth of neurons into the beam. The lasers were so effective that they even induced changes of direction of up to 90º.

In conducting the research, Ehrlicher's team had to choose carefully the intensity of the beam used. 'The power of our laser [was] chosen so that the resulting gradient forces [were] sufficiently powerful to bias the actin polymerization-driven lamellipodia extension, but too weak to hold and move the growth cone,' they explained. By holding and moving cells using optical tweezers, past work has run the risk of causing physical damage to neurons. But according to the scientists their method used light to control a natural biological process.

Armed with these findings, researchers might now be able to produce neural networks interfaced with semiconductors, creating implantable medical devices for neurological disorders.

7 Ehrlicher, A. *et al.* (2002) Guiding neuronal growth with light. *Proc. Natl. Acad. Sci. U.S.A.* 10.1073/pnas.252631899 (http://www.pnas.org)

p53 inhibitors protect the brain

p53 inhibitors have shown promising results in protecting the brain against sudden damage from stroke, and could be used to treat other neurodegenerative diseases, according to a recent report [8]. Current drugs used to treat chronic neurodegenerative conditions, such as Alzheimer's disease (AD) and Parkinson's disease (PD), only provide temporarily relief of symptoms. However, p53 inhibitors could prevent the death of brain cells and thus might have potential as therapies for such diseases.

p53 is a common protein that triggers the biochemical cascade of events leading to cell death. Usually, new, healthy cells are produced to replace the dying cells, but in the diseased or injured brain, cell death can result in devastating damage because brain cells cannot regenerate. However, if p53 was inactivated temporarily, it might be possible to prevent further brain damage. Nigel H. Greig, chief investigator of the study, said: 'By turning off cell death, you rescue brain cells from lethal insult'. He compares other neurodegenerative drugs to 'bandages' that help alleviate brain damage after it occurs, whereas p53 inhibitors act as 'seat belts' that help prevent damage from occurring in the first place.

In the study, synthetic analogues of pifithrin-α (PFT), a p53 inhibitor, were tested for their ability to protect brain cells from damage. *In vitro* studies demonstrated that brain cells given PFT survived longer than those that did not when exposed to various toxic chemicals. In *in vivo* stroke models, PFT significantly reduced the severity of brain damage in animals compared with those that did not receive PFT.

If the p53 inhibitors prove safe, they could be extended to treat AD, PD and amyotrophic lateral sclerosis. Greig and his team are now testing various drug analogues and they hope to begin human trials soon.

8 Zhu, X. *et al.* (2002) Novel p53 inactivators with neuroprotective action: syntheses and pharmacological evaluation of 2-imino-2,3,4,5,6,7-hexahydrobenzothiazole and 2-imino-2,3,4,5,6,7-hexahydrobenzoxazole derivatives. *J. Med. Chem.* 45, 5090–5097

Miscellaneous

fMRI reveals differences between human and monkey brains

Using a standard imaging technique, researchers can now compare directly the brains of humans with those of monkeys, providing evidence, for the first time, that a functional difference exists between them [9].

Functional magnetic resonance imaging (fMRI) measures the volume and flow of blood and also the blood-oxygen levels in the brain. Because neurones need increased amounts of oxygen when they are active, fMRI can also be used as an indirect measure of neuronal activity, by

measuring blood-oxygen levels in different areas of the brain.

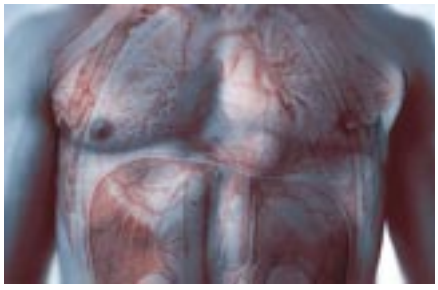
Researchers led by Wim Vanduffel (Athinoula A. Martinos Center for Biomedical Imaging; http://www.nmr.mgh.harvard.edu/index.html) used fMRI to look for differences between humans and macaques in the activity levels in the visual cortex, the region of the brain that processes vision and movement. They recorded patterns of activity in the visual cortex while the subjects were shown rotating 3D images, and found that there were striking differences between the two species.

The differences were most striking in the V3A area of the cortex, which is thought to be involved in several visual functions, including stereoscopic depth and motion processing. There were four areas of the intraparietal cortex that were involved in processing the rotating 3D image in the human subjects, but no clear equivalent was seen in the macaque brains. Although this suggests that human brains might have evolved to control specific abilities, such as controlling fine motor skills, it does not suggest that macaques are unable to process 3D images.

These differences are important because the monkey cortex is often used as a good model for the human cortex, particularly in the neuroscience research community. It is now crucial to determine when it is suitable to use a monkey brain model. The results will also be helpful in extrapolating findings from monkeys to humans much more precisely.

9 Vanduffel, W. *et al.* (2002) Extracting 3D from motion: differences in human and monkey intraparietal cortex. *Science* 298, 413–415

Lacidipine: a multi-talented treatment for cardiac disease?



New results suggest that lacidipine, a Ca^{2+}-channel antagonist known to reduce blood pressure by relaxing cardiac muscle, could also be used to prevent the narrowing of arteries [10].

Both beta-blockers and Ca^{2+}-channel antagonists are used to treat high blood pressure. Beta-blockers, such as atenolol, reduce the tendency of the heart rate to increase when the heart is weakened, whereas Ca^{2+}-channel antagonists relax the heart by limiting the availability of Ca^{2+} in cardiac muscle cells.

The European Lacidipine Study on Atherosclerosis (ELSA), led by Alberto Zanchetti of the University of Milan (http://www.unimi.it/engl/), compared the effects of the Ca^{2+}-channel antagonist lacidipine with those of atenolol, using 2334 hypertensive patients from across Europe. Ultrasound scanning of carotid arteries revealed that lacidipine was significantly more effective at slowing the increase in intima-media thickness (IMT), an index of atherosclerosis, than atenolol. Regression of arterial fatty plaques was also significantly more common in the lacidipine-treated patients. This was despite the findings that the drugs had comparable effects on clinically measured blood pressure and that lacidipine did not reduce ambulatory blood pressure as effectively as atenolol.

These important results provide new insight into the effects of treatments for high blood pressure. They indicate that 'it is some specific property of the calcium antagonist we have used in addition to the lowering of blood pressure, that slows down the progression of atherosclerosis', explains Zanchetti. He suggests that lacidipine could slow the development of plaques by limiting the growth of cells of the arterial wall, possibly being 'more powerful than other calcium antagonists'.

10 Zanchetti, A. *et al.* (2002) Calcium antagonist lacidipine slows down progression of asymptomatic carotid atherosclerosis. *Circulation* DOI: 10.1161/01.CIR.0000039288.86470.DD

Double trouble for tumour angiogenesis

By combining two commonly tried strategies for targeting tumours, a new DNA vaccine eliminates some of the problems associated with the parent techniques [11]. Ralph Reisfeld and co-workers at The Scripps Research Institute (http://www.scripps.edu/) have conducted preclinical studies in which the immune system is stimulated to recognize markers of angiogenesis and recruit killer T cells to destroy the blood vessels.

One strategy for combating cancer is active immunotherapy, in which the immune system is recruited to selectively target and destroy tumour cells. Antigenic markers that are specific or overexpressed in tumour cells can be targeted by T-cells that have been presented with the marker. However, this method is hampered by the diversity of tumour cells: a marker that is overexpressed on one type of cell might not be found on others, rendering the technique unsuitable for broad application. This problem is compounded by the ability of tumour cells to mutate and acquire resistance to T cells by down-regulating the target antigen. A second technique is to block angiogenesis, the process by which a tumour forms new blood vessels to gain nutrients and oxygen. Unfortunately, there are many ways in which a tumour can initiate angiogenesis, and blocking them all is problematic.

Reisfeld and his team have united these two techniques by targeting T cells to endothelial cells that proliferate during angiogenesis. A vaccine approach was developed using a DNA sequence that codes for VEGF receptor-2, a marker that is upregulated on endothelial cells, especially those undergoing angiogenesis around tumours. The antigenic DNA is inserted into a bacterial vector that transports the cargo to the Peyer's patches, which are lymph nodes in the gut. Here, the DNA is released during the breakdown of bacteria. The DNA is taken up by antigen-presenting dendritic cells and macrophages, where it is expressed and presented to T cells. The T cells are thus primed to circulate throughout the body, targeting tumour-supporting endothelial cells that display VEGF receptor 2. 'We hope that these studies established a proof of concept that may eventually contribute to the development of novel cancer therapies,' commented Andreas Niethammer, a co-author of the study.

11 Niethammer, A.G. *et al.* (2002) A DNA vaccine against VEGF receptor 2 prevents effective angiogenesis and inhibits tumor growth. *Nat. Med.* 10.1038/nm794 (http://ww.nature.com)

News in brief was written by *Matt Brown, Peter Chan, Carrie Viccars, Catherine Wild and Heather Yeomans*