News in brief

Targets and mechanisms

Scientists induce state of suspended animation in zebrafish embryo

Scientists have induced suspended animation in a vertebrate, it was reported recently[1]. Researchers from the Fred Hutchinson Cancer Research Center (Seattle, WA, USA) found that embryos of the zebrafish, *Danio rerio*, can survive for 24 h in the absence of oxygen. Upon oxygen deprivation, the fish entered a state of suspended animation, in which all microscopically observed movement ceased, that is, there was no evidence of cell division, development or motility.

In embryos that had developed a heartbeat before oxygen deprivation, researchers could not detect any heartbeat during anoxia until the animals were returned to 'normoxia' (20.8% O_2), at which point they resumed a normal course of development.

This research could shed light on two areas of biology that perplex scientists: the control of stem-cell division and how oxygen deprivation affects tumour growth. Mark Roth, co-author of the report, explains: 'We typically think of cancer cells as growing out of control. The vast majority of cells in a tumour are in a state of low oxygen tension and are non-proliferating, which is the reason why some tumours don't respond to chemotherapy or radiotherapy.'

Stem cells are self-renewing and have the capacity to proliferate at different times of life. To do this, certain populations of cells withhold their proliferation potential by becoming quiescent. There is great scientific interest in how this quiescence is achieved.

Many animals have natural states of suspended animation, for instance to increase reproductive fitness. Roth and colleagues now aim to dissect the molecular pathway that allows this recovery from anoxia. In the future, control over this process could prevent ischaemic injury, allow bodies or organs held in suspended animation to be repaired, and lead to treatments for cancer.

1 Padilla, P.A. and Roth, M.B. (2001) Oxygen deprivation causes suspended animation in the zebrafish embryo. *Proc. Natl. Acad. Sci. U. S. A.* 98, 7331–7335

Small blood vessels are target of chemotherapy

The gastrointestinal (GI) syndrome associated with cancer chemotherapy and radiotherapy is caused by microvascular endothelial apoptosis, researchers have found[2]. GI tract damage has long been the dose-limiting factor for cancer treatment. Researchers at Memorial Sloan-Kettering Cancer Center (New York, NY, USA) have now demonstrated that apoptosis in small blood vessels leads to the stem-cell dysfunction that initiates the effects observed in GI syndrome.

By blocking a cell-signaling pathway called the acid sphingomyelinase pathway – either by a gene mutation or using fibroblast growth factor – GI-tract damage in mice could be prevented. The researchers demonstrated that by blocking this pathway, mice could be given higher doses of radiation therapy with no GI damage.

These findings have great implications for understanding how radiation affects normal organs and tumours, and could provide new approaches for targeting and destroying tumours more effectively. It is hoped that this will be applicable to human cancers, especially those that are treated with whole-body irradiation, such as GI, genitourinary and gynecological tumors.

Although the research only shows that microvascular endothelial cells are targeted in healthy tissue, it is presumed that the same therapies will target the blood vessels in tumours. Richard Kolesnick, senior author of the report and head of the Signal Transduction Laboratory at Memorial Sloan-Kettering said: 'The question we need to ask is whether we will be able to use this new knowledge to protect the blood vessels of healthy tissue or to more effectively target the blood vessels of tumour tissue.'

2 Paris, F. *et al.* (2001) Endothelial apoptosis as the primary lesion initiating intestinal radiation damage in mice. *Science* 293, 293–297

Light shed on mechanism of Alzheimer's disease

Amyloid precursor protein (APP), the precursor of the molecules that cause amyloid plaques to build up in the brains of Alzheimer's disease (AD) patients, might have a role in normal gene expression. APP is a widely expressed cell-surface protein that is embedded in the cell membranes of neurons in the brain. It is cleaved into smaller pieces by a series of enzyme-catalyzed reactions. The last reaction in the series is the cleavage in the transmembrane region by γ-secretase, which results in the plaque-forming extracellular amyloid β-peptide, and the release of an intracellular tail fragment. In their study using cultured cells, Xinwei Cao and Thomas C. Südhof propose that the cytoplasmic APP tail enters the nucleus and participates in gene expression[3].

The researchers hypothesized a similarity between the proposed function of the tail fragment of APP and that of Notch, which is present in the same part of the cell as APP and is cleaved by an enzyme related to γ-secretase. They discovered that APP forms a multimeric complex with the nuclear adaptor protein, Fe65, and the histone acetyltransferase, Tip60. Using binding domains that activate genes to

Regulatory affairs

Wyeth-Ayerst denied pre-market approval for bone regrowth product

The Food and Drug Administration has denied Wyeth-Ayerst Laboratories (Madison, NJ, USA) pre-market approval for their bone regrowth product rhBMP-2/ACS, it was announced recently. The letter that denied the approval focused on the design of the clinical study employed and the interpretation of data from that study.

'We will continue to support this product because we believe in its potential to improve the lives of those who sustain long-bone fractures,' said Bruce Burlington, Senior Vice-President of Worldwide Regulatory Affairs and Compliance at Wyeth-Ayerst Laboratories.

rhBMP-2 is a recombinant version of a protein that naturally stimulates new bone growth in the human body. Wyeth-Ayerst's product combines this protein on an Absorbable Collagen Sponge (ACS) matrix.

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encode fluorescent proteins, they deduced that the complex stimulates transcription via two DNA-binding domains. The results strongly suggest that the cytoplasmic tail of APP functions in gene expression and that the regulation of APP cleavage will not only control release of the cytoplasmic tail, but also the secretion of amyloid β-peptides. The authors suggest that AD is a long-term change in the regulation of amyloid β-peptide – if the pathway is upregulated over a long period of time, more amyloid β-peptide will be produced. This raises the possibility of unwanted side effects from drugs intended to prevent plaque formation that act by interfering with transcriptional regulation. It is still not known which gene is the APP tail's actual target or how APP cleavage is regulated, but this research has shed light on the mechanisms that lead to AD.

In another study[4], it was suggested that Phenserine (Axonyx's lead AD drug, currently in Phase II trials) inhibits the production of amyloid β-peptide. It appears that the drug reduces amyloid β-peptide levels by regulating β-APP via a putative iron-responsive element in the 5′ untranslated region of β-APP mRNA (which has been shown previously to be upregulated by interleukin-1). The mechanism of amyloid β-peptide suppression seems to be independent from the ability of the drug to inhibit acetylcholinesterase, which indicates that Phenserine might not only improve the symptoms of AD, but also interfere with the progression of the disease.

3 Cao, X. and Südhof, T.C. (2001) A transcriptively active complex of APP with Fe65 and histone acetyltransferase Tip60. *Science* 293, 115–120

4 Shaw, K.T. *et al*. (2001) Phenserine regulates translation of β-amyloid precursor protein mRNA by a putative interleukin-1 responsive element, a target for drug development. *Proc. Natl. Acad. Sci. U. S. A.* 98, 7605–7610

HAART therapy avoids HIV-drug resistance

Low levels of HIV-1 virus in the blood of children and adults undergoing a common combination drug therapy (highly active antiretroviral therapy; HAART) do not necessarily indicate that the virus is becoming drug-resistant[5]. Scientists from Johns Hopkins Children's Center and two other institutions aimed to determine the genetic resistance of low-level plasma HIV-1 in patients with prolonged viral suppression while receiving HAART.

A patient can have different variants of HIV-1 because the original infection usually involves many non-identical viruses. The team looked for the unique genetic signatures left by very low levels of HIV-1 and compared each viral genetic sequence with (1) other viral genetic sequences from the same patient; (2) those from other patients; and (3) sequences of drug-resistant viruses. They found that the bloodstream signatures of HIV-1 did not suggest that the virus was developing resistance and thus viral replication and mutation remains suppressed by HAART. This finding has implications for the design and future treatment strategies in fighting the onset of AIDS.

The report also shows that occasional increases in apparent virus activity do not necessarily indicate that the virus is resistant to the protease inhibitors and antiretroviral drugs that comprise HAART. The authors conclude that low-level viraemia in patients receiving HAART with prolonged suppression of viral load might be the result of archival, pre-HAART virus from earlier treatment conditions. The archival drug-resistant virus, however, could be relevant regarding future treatment strategies.

5 Hermankova, M. *et al.* (2001) HIV-1 drug resistance profiles in children and adults with viral load of <50 copies/ml receiving combination therapy. *J. Am. Med. Assoc.* 286, 196–207

Markets

New biologic drugs expected to satisfy unmet psoriasis need

Biologic drugs launched over the next ten years will satisfy the unmet needs of the psoriasis treatment market, claims a recent Decision Resources (Waltham, MA, USA) report[13].

Sales of psoriasis-related biologic agents are expected to generate >US$373 million in 2005 and ~US$923 million in 2010 in the major markets, but particularly in the USA. The need for effective treatments is deemed greatest for moderate to severe cases of psoriasis and psoriasis arthritis (PsA).

Two classes of biologic drugs, cell adhesion molecule (CAM) antagonists and tumour necrosis factor (TNF) inhibitors, are expected to drive the market expansion. Two CAM antagonists, alafacept (Biogen's Amevive) and hull24 (Genentech/Xoma's Xanelim), are expected to be launched by early 2003. The increased prescription of TNF inhibitors by rheumatologists for PsA is expected to hasten their acceptance by psoriasis sufferers because PsA represents a significant proportion of the 10 million psoriasis cases in Europe, the UK and the USA.

13 Sharma, D. and Tankosic, T. (2001) *Psoriasis and Associated Arthropathy*. Study 35, 1–121 (available at http://www.dresources.com)

Chink in armour of Ebola and Marburg viruses

Ebola and Marburg viruses might use folate receptors (FRs) to infect cells, it was reported recently[6] by researchers at the Gladstone Institute of Virology and Immunology (University of California at San Francisco; UCSF, CA, USA).

Genes from cells susceptible to the viruses were randomly inserted into cells known to be naturally resistant against the viruses. When the cells were exposed to virus, only cells with the gene encoding FR-α were infected. FR-α has been shown to facilitate virus entry in Jurkat cells and, furthermore, FR-antagonists (FR-α specific antibodies and high concentrations of folic acid) inhibited infection by either virus. In another study, it was demonstrated that cells expressing Marburg and Ebola surface glycoproteins bound to FR-α, and mediated syncytia formation.

These findings reveal FR-α to be a significant cofactor for viral infection. 'It could stop the virus dead in its tracks before it has an opportunity to multiply,' said Mark Goldsmith, Associate Investigator at Gladstone and Professor of Medicine at UCSF.

'However,' warns Goldsmith, 'it is strongly suspected that the viruses also use other receptors to gain entry to cells.'

6 Chan, S.Y. *et al*. (2001) Folate receptor-α is a cofactor for cellular entry by Marburg and Ebola viruses. *Cell* 106, 117–126

Enhanced HSV-1 kills cancer cells more effectively

A variant of herpes simplex virus-1 (HSV-1) that has been engineered to replicate more efficiently is better at killing cancer cells, it was reported recently[7]. HSV-1 strains with mutations in the virulence gene γ34.5 have been shown to reduce the growth of human tumour xenografts in mice and have passed Phase I safety studies, but their efficacy has been hindered by poor virus replication.

Scientists at New York University School of Medicine (New York, NY, USA) have previously identified a γ34.5 deletion mutant that retains the ability to replicate in tumour cells, while still being attenuated[8]. In this recent report[7], the authors demonstrate that the mutated virus replicates to greater levels in prostate carcinoma cells. More important, however, was the finding that this variant is a more effective antitumour agent in an animal model of human prostate cancer than the γ34.5 parent virus. The authors believe this increased therapeutic potency could prove useful for several types of cancer.

7 Taneja, S. *et al*. (2001) Enhanced antitumor efficacy of a herpes simplex virus mutant isolated by genetic selection in cancer cells. *Proc. Natl. Acad. Sci. U. S. A.* 98, 8804–8808

8 Mohr, I. *et al.* (2001) A herpes simplex virus type 1 γ34.5 second-site suppressor mutant that exhibits enhanced growth in cultured glioblastoma cells is severely attenuated in animals. *J. Virol.* 75, 5189–5196

Miscellaneous

New gene variants crucial for personalized medicine

Genaissance Pharmaceuticals (New Haven, CT, USA) have announced details of the most extensive examination of human genes ever conducted[9]. The findings of the study, which investigated variability within genes, could provide the basis for new personalized medicines.

The research identified 3899 single nucleotide polymorphisms (SNPs) present within 313 genes from 82 unrelated individuals. The SNPs were organized into 4304 different haplotypes. Given that there are ~30,000 human genes, the results indicate that the human genome comprises some 500,000 gene versions.

Genaissance believe that the safety and side-effect profile of a drug in an individual depends largely on the versions of genes that each person has inherited. The company has already demonstrated that a response to a drug can be predicted from an individual's DNA using its proprietary HAP™ Technology[10].

'These studies will cause the scientific and medical communities to rethink the definition of the human genome,' said Gualberto Ruano, CEO of Genaissance. 'We found that there are, on average, 14 versions of each gene that can be inherited by a human being. By being able to define gene versions, we can now determine those versions that predict drug response and, hence, change the current paradigm that one drug fits all individuals.'

9 Stephens, J.C. *et al.* (2001) Haplotype variation and linkage disequilibrium in 313 human genes. *Science* 10.1126/science. 1059431 (http://www.sciencemag.org/sciencexpress/recent.shtml)

10 Drysdale, C.M. *et al.* (2000) Complex promoter and coding region β2-adrenergic receptor haplotypes alter receptor expression and predict *in vivo* responsiveness. *Proc. Natl. Acad. Sci. U. S. A.* 97, 10483–10488

Privately funded stem cell research highlights need for federal oversight

US scientists have used sperm and eggs specifically donated for use in research to create new embryonic stem cell lines, the Coalition for the Advancement of Medical Research (Washington, USA) announced recently.

In the study[11], excess fertilized eggs from *in vitro* fertilization procedures were used to generate human embryonic cell lines for use in stem cell research. The researchers report that they ensured that oocyte and sperm donors understood the nature and purpose of the research before participating in the study. However, the privately funded research, performed at the Jones Institute for Reproductive Medicine at Eastern Virginia Medical School (Norfolk, VA, USA), would not have been permitted under current federal guidelines.

The NIH is currently deciding whether or not to allow the federal funding of this type of research in the future, and is expected to make a decision soon. 'This research demonstrates the urgent need for federal oversight,' said Lawrence Soler, Chairman of the Coalition for the Advancement of Medical Research, 'but federal oversight will only come hand-in-hand with federal funding.'

11 Lanzendorf, S.E. *et al.* (2001) Use of human gametes obtained from anonymous donors for the production of human embryonic stem cell lines. *Fertil. Steril.* 76, 132–137

NMR on the move

High-resolution nuclear magnetic resonance (NMR) spectroscopy using a strongly non-uniform magnetic field has been made possible, enabling the development of mobile scanning equipment, it was reported recently[12].

'It may be possible to develop a mobile magnet that can be scanned over otherwise inaccessible objects,' said Carlos Meriles, Professor of Chemistry at the University of California at Berkeley (CA, USA).

NMR spectroscopy has previously been carried out by exposing samples to a strong, uniform magnetic field. The large immobile magnet used causes nuclei within a sample to magnetically align. When irradiated by a specific radiowave pulse, the nuclei 'wobble' on the field axis, generating atom-specific data that can be used to define which elements are present.

Producing mobile magnets has been hindered in the past by the nature of the non-uniform field used; small differences in magnetic charge produced by the wobble cannot be read because they are overwhelmed by much larger variations in non-uniform magnetic field.

These problems have been overcome in this study by using a combination of three radiowave pulses of varying radiowave frequencies rather than just one, explained Meriles and his colleague Alexander Pines, of the Materials Sciences Division of Ernest Orlando Lawrence Berkeley National Laboratory (Berkeley, CA, USA). The three radiowave pulses knock the nuclei, such that the characteristic NMR data can be recovered with virtually the same sharp resolution as with a uniform magnetic field.

12 Meriles, C.A. *et al*. (2001) Approach to high-resolution *ex situ* NMR spectroscopy. *Science* 293, 29382–29385

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