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News in brief

Targets and mechanisms

First HIV-1 transgenic rat

Researchers in the USA have engineered the first HIV type 1 (HIV-1) transgenic rat model[1]. The model was engineered by scientists at the University of Maryland Biotechnology Institute (UMBI; Baltimore, MD, USA) and contains the genome of HIV-1, with functional deletion of the *gag* and *pol* genes, thus rendering the virus non-infectious.

The rats were extensively studied for molecular, immunological and histopathological characteristics of HIV infection, as well as their general health. Spliced and unspliced HIV transcripts were identified in the rat lymphatic organs: lymph nodes, thymus, kidney and spleen, which suggests that the *Tat* and *Rev* genes of the virus are functional.

Immunohistochemistry was used to detect viral proteins in tissue sections of the spleen, and HIV glycoprotein gp120 (the protein that initiates HIV entry via CD4 expressed on host-cell membranes) was isolated in splenic macrophages, T and B lymphocytes and serum. Further evidence of infection included increased apoptosis of endothelial cells and splenocytes, lymphocyte depletion, interstitial pneumonia, psoriasis, and neurological, cardiac and renal pathologies.

By 5–9 months of age, the rats had symptoms similar to those of humans with AIDS, including wasting, mild–severe skin lesions, opaque cataracts, neurological symptoms and respiratory difficulties. Thus, the transgenic rat has many similarities to humans infected with HIV-1.

Joseph L. Bryant, Head of the Animal Model Division of UMBI's Institute of Human Virology, comments that: 'The new HIV transgenic rat is an excellent model for studying early and chronic infections, that is, the tracking of the clinical cellular and immunologic course of HIV-1 in humans. It will be especially effective in testing potential therapeutic strategies against chronic AIDS-associated conditions or syndrome.'

1 Reid, W. *et al*. (2001) An HIV-1 transgenic rat that develops HIV-related pathology and immunologic dysfunction. *Proc. Natl. Acad. Sci. U. S. A*. 98, 9271–9276

A bowel movement a day keeps Parkinson's away

Men who have infrequent bowel movements are more likely to develop Parkinson's disease (PD) in later life, concludes a recent article in the journal *Neurology*[2]. Having less than one bowel movement per day was associated with a 2.7-fold greater risk of developing the disease, when compared with men who had an average of one bowel movement per day. This risk increased to 4.1-fold when compared with men that had two bowel movements per day and 4.5-fold for those that had more than two.

Information was collected from 6790 men without PD, aged 51–75, participating in the Honolulu Heart Program (Oahu, HI, USA). Over the next 24 years, 96 participants developed PD. The results were adjusted to take into account differences in age, smoking, coffee consumption, laxative use, jogging, and intake of fruits, vegetables and grains but the tie between bowel movement frequency and PD remained.

The group, led by Robert D. Abbott from the University of Virginia School of Medicine (Charlottesville, VA, USA) and Pacific Health Research Institute (Honolulu, HI, USA) noted that further study is required to determine whether constipation is part of early PD or a marker of susceptibility or environmental factors that might cause PD. However, he suggested that 'The same processes that cause the motor symptoms of Parkinson's may also affect the colon's functioning. There may also be some abnormalities in the muscles involved in bowel movements.'

This could, therefore, be an important finding because it could help understand how the disease progresses. Abbott therefore suggested that 'It could help us more effectively identify people with early or suspected disease or people at high risk of developing the disease in the future.'

2 Abbott, R.D. *et al.* (2001) Frequency of bowel movements and the future risk of Parkinson's disease. *Neurology* 57, 456–462

Human cloning could lack problems associated with cloning non-primates

Humans could be easier to clone than non-primates because of differences in the way in which the gene for the insulin-like growth factor II receptor (IGF2R) is inherited[3]. Researchers from Duke University Medical Center (Durham, NC, USA) showed that genomic imprinting, where one *IGF2R* gene is turned off, greatly increases the chance of cloned animals being born with defects, such as overly large offspring development, immature lung development, enlarged hearts and reduced immunity to disease. The reason behind why turning one gene off has this effect is not understood. However, the process is absent from primates and their closest non-primate relatives but is common in non-primate mammals such as mice, rats, sheep, pigs and cows.

One theory is that the very state of being imprinted increases the chance of epigenetic damage being caused when embryos are being prepared in the laboratory, said Randy Jirtle, Professor of Radiation Oncology at Duke University. Keith Killian, first author of the paper[3] said: 'Only one in 300 sheep embryos take hold and up to half of these embryos have large offspring syndrome, which can kill the mother and the fetus. Since humans are not imprinted at *IGF2R*, then fetal overgrowth would not be predicted to occur if humans were cloned.'

Killian and his colleagues employed six different single nucleotide polymorphisms to test for genomic imprinting. It was shown that *IGF2R* has not been imprinted in mammals for approximately 70 million years. 'Knowing where on the evolutionary scale *IGF2R* imprinting appeared and subsequently vanished will enable scientists to select animal models better suited to making inferences about human clonability and cancer susceptibility,' said Jirtle. 'If you don't know animals are related to each other, there is no way to accurately extrapolate the experimental results from one species to another.'

Many scientists have believed that up to 50% of people are imprinted at the *IGF2R* gene. However, using gene mapping technology, Killian and colleagues found no evidence that any humans possess an imprinted *IGF2R*. Jirtle pointed out that as humans and other primates have two activated copies of the gene, whereas most non-primates only have one copy, mutation of only one copy of the gene in mice and other imprinted animals that can lead to various forms of cancer means that they are far more susceptible to cancers than the

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mutation of the two copies necessary to cause cancers in humans. Killian highlights the importance of taking into account the rodents' susceptibility to cancer when they are applying their study conclusions to humans. He therefore points out that 'You could theoretically give new life to thousands of discarded compounds by retesting them in animals that, like humans, have both functional copies of *IGF2R*.'

3 Killian, J.K. *et al.* (2001) Divergent evolution in *M6P/IGF2R* imprinting from the Jurassic to the Quaternary. *Hum. Mol. Genet.* 10, 1721–1728

Promising vaccine for Alzheimer's disease

Scientists have successfully prevented the development of Alzheimer's disease by using a new vaccine that they suggest should be safer than one already being tested in early human clinical trials. In a recent study[4], researchers at New York University (NYU) School of Medicine (Staten Island, NY, USA) used transgenic mice that were engineered to develop amyloid-β (Aβ) plaques in the brain, and immunized them with a soluble, non-amyloidogenic non-toxic Aβ-homologous peptide.

Their work followed previous studies that demonstrated reduced cerebral amyloid burden in mice immunized with aggregated Aβ1–42 peptide[5]. However, the use of this peptide can cause toxic fibrils and so the group at NYU have been investigating the use of non-fibrillar alternatives.

They found that immunization of transgenic mice (Tg2576) for seven months with a non-toxic homologue of Aβ1–42 reduced the cortical and hippocampal brain-amyloid-burden by 89% and 81%, respectively, compared with transgenic mice that had not been administered with the vaccine. Furthermore, brain levels of soluble Aβ1–42 were reduced by 57%, and ramified microglia expressing interleukin-1β associated with Aβ plaques were absent in the immunized mice.

Thomas Wisniewski, Associate Professor of Neurology, Pathology and Psychiatry at NYU, says, 'Our study clearly shows that the vaccination approach is a powerful one that shows great promise for Alzheimer's disease and, significantly, our approach appears to be non-toxic.' Wisniewski thinks this might be because the non-fibrillar peptide is soluble and does not form clumps.

Early clinical trials of the new vaccine could begin within one year.

4 Sigurdsson, E.M. *et al.* (2001) Immunization with a nontoxic/nonfibrillar amyloid-β homologous peptide reduces Alzheimer's disease-associated pathology in transgenic mice. *Am. J. Pathol.* 159, 439–447

5 Morgan, D. *et al.* (2000) Aβ peptide vaccination prevents memory loss in an animal model of Alzheimer's disease. *Nature* 408, 982–985

Positive results for cardiovascular gene therapy

A novel cell-cycle-inhibitor fusion gene, *p27–p16*, has demonstrated potent inhibition of coronary artery occlusion that results from restenosis[6] (re-narrowing of the blood vessels following angioplasty). The preclinical trial is part of a collaboration between Cell Genesys (Foster City, CA, USA) and GPC Biotech (Münich, Germany) to evaluate GPC Biotech's proprietary fusion gene for its antiproliferative properties. The studies showed up to 60% reduction of angioplasty-induced coronary intimal artery thickening in blood vessels after treatment with the *p27–p16* gene therapy.

The gene was created by fusing the active regions of p27 and p16 cell cycle inhibitors. A replication-deficient adenoviral vector (AV) encoding this p27–p16 fusion protein was delivered to porcine coronary arteries using an infusion catheter. The *p27–p16* gene therapy demonstrated significant inhibition of smooth muscle cell proliferation (responsible for the narrowing of the blood vessels) compared with untreated animals, with no observed toxicities.

In several preclinical models, the *p27–p16* fusion gene has demonstrated greater antiproliferative activity than either of the parental genes, *p16* or *p27*. The studies suggest that the gene fusion therapy could be useful in preventing angioplasty-induced intimal hyperplasia.

6 Tsui, L.V. *et al.* (2001) *p27–p16* fusion gene inhibits angioplasty-induced neointimal hyperplasia and coronary artery occlusion. *Circ. Res.* 189, 323–328

Clinical trials

Clinical trials of cannabis extracts begin in Canada

Clinical trials of medicines derived from cannabis are to begin in Canada, announced GW Pharmaceuticals (Salisbury, Wiltshire, UK) recently. The trials, which will involve cannabis extract administered by a sublingual spray, has been made possible by the Canadian regulatory authority, Health Canada, bestowing an Investigational New Drug grant to GW earlier this year.

The randomized, double-blind, Phase II trial will take place at The Rehabilitation Center, Ottawa Hospital (Ontario, Canada), and will include sufferers of multiple sclerosis, spinal cord injury and other forms of chronic pain. GW Pharmaceuticals began Phase II trials on cannabis extracts in Europe in May 2000 and is currently recruiting for a Phase III trial for multiple sclerosis in the UK. However, the company claims that this Canadian trial is the only trial of its kind in North America.

The company report that the results of previous trials have shown a clear benefit to patients taking the treatment, with clinically significant improvements in a range of symptoms including pain, muscle spasms, spasticity, bladder-related symptoms, tremor and overall improvements in quality of life.

Cancer targets and mechanisms

Role for PDGFR-α in basal cell carcinoma

Platelet-derived growth-factor receptor-α (PDGFR-α) has a role in the proliferation of the most common human cancer, basal cell carcinoma (BCC), a recent study has revealed[7]. Researchers at the University of California (San Francisco, CA, USA) have investigated the activation of the Sonic hedgehog pathway (involved in organism

development), which is a frequent event in the progression of BCC.

Upon arrival at the cell surface, hedgehog launches a series of chemical reactions. A mutation either in the membrane protein that binds to hedgehog at the cell surface (known as patched or PTC), or in the next protein in the cascade called smoothened (SMO), can result in the hedgehog pathway being constitutively activated. Furthermore, a downstream protein in the hedgehog pathway, Gli1, is upregulated in BCC and has been shown to accelerate the progression of BCC when overexpressed in mouse epidermis[8].

Jingwu Xie and colleagues have now shown that Gli1 can activate PDGFR-α in C3H10T(1/2) fibroblast cells[7]. This was shown to be concurrent with activation of the Ras–extracellular regulated kinase (ERK) pathway, which has long been associated with the regulation of cell division. Inappropriate regulation of PDGFR-α and, thus, the Ras–ERK pathway could, therefore, result in skin cancer. The activation of Gli1 is further supported by *in vivo* data that show high-level expression of PDGFR-α in BCCs of mice and humans. Moreover, suppression of PDGFR-α expression by *PTC* gene overexpression or antibody neutralization reduces the level of DNA synthesis and proliferation in a murine BCC cell line (ASZ001). Jingwu Xie believes that this could lead to the first drug therapy, perhaps in a cream or sunscreen, that could stop the most common form of cancer that afflicts humans.

7 Xie, J. *et al*. (2001) A role of PDGFR-α in basal cell carcinoma proliferation. *Proc. Natl. Acad. Sci. U. S. A.* 98, 9255–9259

8 Dahmane, N. *et al*. (1997) Activation of the transcription factor Gli1 and the Sonic hedgehog signalling pathway in skin tumours. *Nature* 389, 876–881

Potential gene markers identified for melanoma

Scientists have discovered how precancerous moles might progress to melanomas, which are the most deadly form of skin cancer. A recent study by researchers at Johns Hopkins University School of Medicine (Baltimore, MD, USA) has identified a transcriptional repressor of a key growth-regulatory gene that is upregulated in early melanomas[9]. The helix–loop–helix transcription factor, Idl, regulates G1–S-phase cell-cycle transitions and has been shown to repress Ets- and E-protein-mediated transactivation of an important tumour suppressor gene, *p16/Ink4a*.

The researchers at Johns Hopkins decided to investigate the repression of *p16/Ink4a* because it is inactivated in several subsets of familial and sporadic melanomas. To this end, they evaluated 21 melanocytic lesions at various stages of progression, from common melanocytic nevi to metastatic melanomas, for expression of Idl and *p16/Ink4a*. Expression of Idl was found to correlate with a loss of *p16/Ink4a* expression in early melanoma *in situ*, in contrast to advanced melanomas, which do not express Idl despite reduced *p16/Ink4a* expression in these regions.

Rhoda Alani, Assistant Professor of Oncology at Johns Hopkins, says, 'Telling the difference between precancerous moles and early stage melanoma can be very difficult. If it's melanoma, you want to catch it very early and treat it aggressively to cure the disease. Because Idl is expressed in early stage melanoma, it has the potential to serve as a definitive diagnostic marker, although more studies are needed to confirm this.'

9 Polsky, D. *et al*. (2001) The transcriptional repressor of *p16/Ink4a*, Idl, is upregulated in early melanomas. *Cancer Res*. 61, 6008–6011

Cell-surface molecule key to breast cancer prevention

A molecule present on the surface of cells is expressed at high levels on breast cancer cells and could lead to better methods for the prevention and treatment of the disease[10]. Glypican-1 is a member of a family of glycosylphosphatidylinositol-anchored cell-surface heparin-sulfate proteoglycans that are implicated in the control of cell growth and differentiation. Researchers at the University of California, Irvine (UCI; Irvine, CA, USA) have shown that this proteoglycan is upregulated on the surface of breast cancer cells compared with normal breast tissues.

Using northern blotting of normal and cancerous breast tissue, the group found that glypican-1 expression was sixfold higher than other members of the glypican family. Levels of glypican-3 and -4 mRNA were only slightly higher in cancerous tissue and levels of glypican-2 and -5 mRNA were below the level of detection in both samples. Moreover, treatment of two breast cancer cell lines with phosphoinositide-specific phospholipase C attenuated the cellular response to heparin-binding growth factors (heparin-binding epidermal growth factor-like growth factor and fibroblast growth factor 2), suggesting that glypican-1 has a pivotal role in the mitogenic activity of breast cancer cells in response to several growth factors.

Arthur Lander, Associate Professor of Developmental and Cell Biology at the UCI School of Biological Sciences, suggests that this role might be mediated via the cancer gene *c-erbB-2*: 'The gene *c-erbB-2* is found to amplify its activities in breast cancer cells. Although we've never found a specific molecular binding partner for *c-erbB-2*, we think that other receptors used by glypicans and similar molecules might assist *c-erbB-2* in its accelerated activity in cancer.' He concluded, 'We need to see if interrupting glypican's activity would result in a reduction in the growth of cancer cells in later stages of cancer, or if glypican could be used as a marker to allow for earlier diagnosis of cancer.'

10 Matsuda, K. *et al*. (2001) Glypican-1 is overexpressed in human breast cancer and modulates the mitogenic effects of multiple heparin-binding growth factors in breast cancer cells. *Cancer Res*. 61, 5562–5569

Miscellaneous

Battle of the supercomputers

Four almost simultaneous new partnerships have been established between drug discovery research companies/organizations and supercomputing companies/centres. A new partnership, bringing together a supercomputing facility with a leader in genomic research, has recently been announced. The Institute for Systems Biology (ISB; Seattle, WA, USA) and the Arctic Region Supercomputing Center (ARSC; University of Alaska, Fairbanks, AK, USA) will support genomic and proteomic research in which extremely large datasets will be analyzed. The ARSC computers will provide the memory, processing power and data storage resources needed to analyze the datasets from ISB's research.

An IT agreement between Entelos (Menlo Park, CA, USA), which specializes in biosimulations for *in silico* drug discovery and development, and Compaq has also been established. Entelos will use Compaq's technology to develop high-throughput simulation to set up a scalable, virtual laboratory to speed up the testing of new drug therapies. The 'Physiolabs' will enable large-scale generation of virtual patients, targets and therapies and the assessment of gene and protein functions. The results will be used by pharmaceutical companies to identify novel pathways, prioritize drug targets, optimize experiments and clinical trials, understand variable patient responses to treatment and increase productivity by avoiding the bottlenecks associated with the traditional drug development pipeline. The two companies will undertake joint marketing activities and provide coordinated customer support throughout the three-year agreement. Compaq AlphaServer systems running Tru64 UNIX were selected to provide the computing capability.

IBM and the biological modelling company Physiome Sciences (Princeton, NJ, USA) have also announced a similar modelling agreement to that of Entelos and Compaq. Physiome Sciences will use IBM's supercomputing technology for research into biological systems, disease and potential drug targets and IBM will license biological modelling technology from Physiome Sciences for its internal use. The supercomputers will enable Physiome Sciences to speed up its current research into simulating complex models of cells, organs and disease states. The company says that pharmaceutical and other biomedical researchers who use Physiome's simulation technology should be able to perform modelling studies quicker and at less cost than at present to gain insights into drug targets and disease mechanisms. IBM and Physiome Sciences will also work to promote open standards such as CellML™ (an XML-based language to develop computer models of cells, tissues and organs).

Finally, Vertex Pharmaceuticals (Cambridge, MA, USA) has installed a state-of-the-art supercomputer dedicated to *in silico* drug design applications. The supercomputer will perform parallel computations using proprietary software and modelling expertise that supports Vertex's various research groups, including bioinformatics and pharmacology. The supercomputer will also provide a tool for analyzing complex data and information generated using cell-based assays and ultra-HTS approaches.

Parallel computation is playing an increasing role in drug discovery because it enables identification and selection of potent lead drug candidates, as well as accelerated screening of large computer-generated libraries, enhanced ability to measure protein–inhibitor interactions *in silico* and increased power to analyze immense datasets.

UCSD Cancer Center achieves 'Comprehensive' status

The Cancer Center of the University of California, San Diego (UCSD; CA, USA) has achieved the status of Comprehensive Cancer Center, the highest ranking that can be bestowed by the US Federal Government. The title signifies that the center performs basic and clinical science, as well as population studies, community outreach programs and cancer-prevention studies, and places it in a grouping with 40 other such centers in the USA. The National Cancer Institute, who awarded the ranking, recently tripled its previous level of support for the UCSD Cancer Centre, providing nearly US$19 million over a five-year period.

Boston University and UC-Davis receive Leadership Awards

Boston University (Boston, MA, USA) and the University of California, Davis (UC-Davis; CA, USA) have both received large monetary awards from The Whitaker Foundation (Arlington, VA, USA). Boston University will use the US$14 million Leadership Award to hire 12 new faculty members, develop new courses, renovate teaching and research space, and purchase new equipment for teaching.

The UC-Davis will use its US$12 million Leadership-Development Award to build a new biomedical engineering building, develop a new undergraduate biomedical engineering program, buy new laboratory equipment, and hire five new faculty members within the next three years.

The Whitaker Foundation Leadership-Development Awards program supports major initiatives to enhance the field of biomedical engineering at research universities in the USA. The awards were made possible by the Foundation's decision to spend all of its resources and close by the end of 2006.

July was a bad month for biotech stocks

Cancelled collaborations, negative Food and Drug Administration (FDA) decisions and poor second quarter earnings caused indices of biotechnology stocks to perform poorly in July, reported Burrill & Company (San Francisco, CA, USA), recently. The Burrill Life Sciences Composite fell by 7% while the Nasdaq also suffered, dropping 6%.

In the month, Titan Pharmaceuticals (South San Francisco, CA, USA) shares almost halved in value following news that a regulatory filing for their schizophrenia drug, Zomaril, would be delayed a year. The company decided to delay the application to allow more patient testing to be done. Aviron (Mountain View, CA, USA) were also the recipient of regulatory problems. One-third was wiped off their share price when an FDA advisory panel voted that their nasal spray vaccine, Flumist, had failed to establish sufficient evidence of safety required for marketing clearance. The product will now miss the 2001–2002 'flu season. Furthermore, Amylin Pharmaceutical (San Diego, CA, USA) suspended trading of their shares after receiving a negative judgement from the FDA regarding their diabetes lead product candidate. At one point, their shares fell by 25%.

The Burrill Select index fell by 11% in July as FDA concerns over the asthma-targeting monoclonal antibody, Xolair, affected antibody technology-based companies. Similarly, Burrill's Genomics index fell by 23%, influenced by declines in the share price of Celera (Rockville, MD, USA), down 23%, and Curagen (New Haven, CT, USA), down 38%.

'This is the volatile nature of the biotech market and, by now, investors should know that each month has its winners and losers. Investors who pick a portfolio of diversified companies… and then cancel their subscription to the *Wall Street Journal*, turn off their computer, skip CNN, and give it time, will be rewarded,' said G. Steven Burrill, CEO of Burrill & Company.

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