News in brief

Viral Targets and Mechanisms

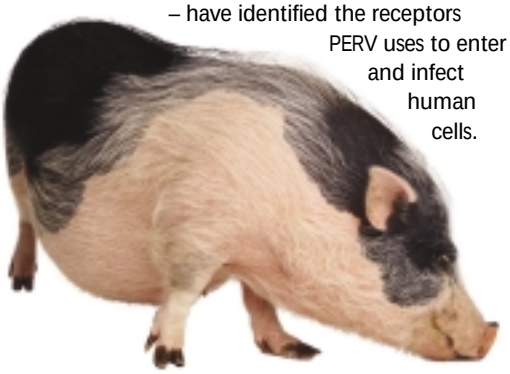
'Farm'acology: locating receptors to pig virus

A recent insight into the biochemistry of a potentially troublesome pig virus has renewed optimism that transplantation of organs from animals to humans might one day become a reality [1].

One potential way to make the supply of organs for transplantation meet the demand is xenotransplantation, the transfer of animal organs into humans. Pigs are considered the most promising donor candidates, mostly because of the similarity in size and layout of their internal organs to those of humans.

However, in addition to issues of organ rejection and intolerance, such operations would run the risk of introducing porcine viruses into the recipient. Porcine endogenous retrovirus (PERV) has been identified as a particular concern. All pigs harbour the virus, which causes no harm to the animal, however, little is known about its mechanisms of infection and the effect it would have on humans. For example, PERV has been tested in 40 human cell lines, of which only three became infected. Why the virus is able to infect human cells, but only a small proportion, remains unexplained.

An important step towards understanding PERV infection has recently been made. Researchers from Immerge BioTherapeutics [a joint venture between Novartis AG (http://www.novartis.com/) and BioTransplant (http://www.bioscorpio.com/biotransplant_inc.htm)] – in collaboration with University College London (http://www.ucl.ac.uk) and The Scripps Research Institute (http://www.scripps.edu/) – have identified the receptors PERV uses to enter and infect human cells.



A cDNA library approach was used to identify two multitransmembrane receptors for PERV-A, the most common form of the virus, and the only type to demonstrably infect human cells. The physiological role of the receptors is unknown, but homologous receptors have been found in the pig and baboon.

Dan Solomon, one of the authors from Scripps commented on the findings: 'It remains important to identify the risk of a potentially infectious agent to both the patient receiving a transplant as well as others. Our identification of the PERV receptor will allow us to begin to address this issue.' Although this is an important early step towards preventing infectivity of PERV in humans, many questions still remain. Why, for example, are most human cells seemingly immune to infection, despite ubiquitous expression of the PERV-A receptor?

1 Ericsson, T.A. *et al.* (2003) Identification of receptors for pig endogenous retrovirus. *Proc. Natl. Acad. Sci. U. S. A.* 100, 6759–6764

Genomics and Proteomics

RNAi silences dominant disease genes

A novel way has been found to selectively suppress a dominantly acting disease gene without affecting the function of the normal copy [2]. The technique could lead to treatments for a wide range of genetic illnesses, including cancers, neurodegenerative diseases and viral infections.

In many inherited diseases, the effects of a mutant copy of a gene dominate those of its wild-type counterpart. The intuitive solution would be to somehow block the dysfunctional gene without affecting the normal gene. This has proven technically complicated, however, and the first successes are only now appearing, spurred on by the recent developments in RNA interference (RNAi). This procedure is an increasingly appreciated method of specifically silencing genes by the introduction of small interfering RNAs (siRNAs).

Researchers at the University of Iowa (http://www.uiowa.edu/) have been using RNAi to specifically target dominant disease-causing genes. Part of their work focused on the neurodegenerative condition called Machado-Joseph disease (MJD), a genetic illness akin to Huntington's disease. In MJD, a genetic defect produces mutated proteins with an abnormally long stretch of repeated glutamine residues. These mutant proteins have a tendency to clump together and damage brain tissue, a common motif in neurodegenerative disease.

Initial attempts to target the mutation with siRNAs were disappointing. The RNA was unable to distinguish between the mutant and normal genes, and both were suppressed. The researchers then decided to target a single nucleotide polymorphism (SNP) close to the polyglutamine mutation. This was effective, knocking out the mutant gene while enabling the wild-type gene to fulfil its normal role. In further studies, the technique was successfully targeted to a disease-causing mutation. RNAi was applied to a mutant Tau protein having only a single base-pair change in the gene, a mutation often found in inherited dementias.

Because the technique can exploit the difference of only one nucleotide, be it an SNP or the disease-causing mutation, the researchers are optimistic. However, Victor Miller, lead author of the paper, stressed that although the work is an important proof of principle, it is still a long way from clinical application.

2 Miller, V.M. *et al.* (2003) Allele-specific silencing of dominant disease genes. *Proc. Natl. Acad. Sci. U. S. A.* 100, 7195–7200

A dangerous world for genome stability

Cadmium, a widely occurring natural element and known carcinogen, could cause harmful genetic defects, not only by damaging DNA directly, but also – as has recently been reported – by inhibiting DNA repair [3].

Researchers at the National Institute of Environmental Health Sciences (http://www.niehs.nih.gov) have conducted extensive studies into the mechanism by which cadmium causes high mutability: they used yeast cells, which match initial results using cultured human cells. The results showed that the mutagen inhibits the ability of cells to repair routine errors made during DNA replication. More specifically, cadmium reduces the capacity for post-replication

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mismatch repair (MMR) of small misalignments and base–base mismatches.

Mutation-avoidance systems, such as DNA polymerase proofreading and MMR are crucial to rectifying mistakes made during the process of DNA duplication. Without these mechanisms, mutations occur and multiply to 'catastrophic' proportions, says Michael A. Resnick, a co-author of the report. Blocking post-replication MMR can increase mutations as much as 2000-fold, potentially leading to cancer, reproductive problems, birth defects and more.

Cadmium occurs naturally in soils and rocks, is used in plastics and batteries, and shows up in cigarette smoke – smoking doubles the average daily intake. Environmentally relevant concentrations of cadmium caused substantial effect in these studies, the amount of metal needed to increase mutations being remarkably small. These studies help to understand the role of environmental factors in genome stability.

3 Jin, Y.H. *et al.* (2003) Cadmium is a mutagen that acts by inhibiting mismatch repair. *Nat. Genet.* Jun 8 (epub ahead of print; http://www.nature.com)

Targets and Mechanisms

Will Popeye develop Parkinson's?

Is high dietary iron linked intake linked to developing Parkinson's disease (PD)? Researchers at the University of Washington School of Medicine (http://www.washington.edu/medicine/som/) have reported findings that show a correlation [4].

The data collected from PD and non-PD control subjects over a ten-year period show that those adults with the highest level (top 25%) of iron in their diets were 1.7-times more likely to be PD patients, compared with subjects whose iron intake was in the lowest (bottom 25%) portion. Also, an apparent joint effect of iron together with manganese – when both were consumed at above average levels – was reported; such that, a 1.9-times increased chance of being a PD sufferer was conferred. The likelihood of being a PD patient was raised even more when the subjects' intake of either iron or manganese was above average and supplemented with multivitamins.

Iron and manganese both contribute to oxidative stress, and 'oxidative stress may cause degeneration of brain cells that produce dopamine – the same cells that are affected by Parkinson's disease', said report author Harvey Checkoway of the University of Washington.

These results by no means suggest that high iron intake causes PD, but instead could improve understanding of how PD develops. The benefits of eating foods rich in iron and manganese outweigh the risks of developing PD, which probably has numerous contributory factors, for example, environment, lifestyle and genetic. Therefore, dietary recommendations for preventing the development of PD are still a long way off.

4 Powers, K.M. *et al.* (2003) Parkinson's disease risks associated with dietary iron, manganese and other nutrient intakes. *Neurology* 60, 1761–1766

Cancer Targets and Mechanisms

PEDF as an anti-tumour agent in human prostate cancer

Researchers from the Feinberg School of Medicine at Northwestern University in Chicago (http://www.nums.nwu.edu), and Umeå University, Sweden (http://www.umu.se), have shown that pigment epithelium-derived factor (PEDF) regulates the blood supply and mass of the prostate and pancreas, providing hope of a new treatment for prostate cancer in humans [8].

Normal prostate glands are lined with a simple columnar epithelial layer. Doll *et al.* created PEDF-deficient mice, which displayed substantial stromal vascularity and epithelial cell hyperplasia. This hyperplasia in PEDF-deficient mice prompted the researchers to examine PEDF levels in human prostate tissue. Normal human prostate tissue expresses PEDF; however, the levels of PEDF in human prostate cancer biopsy tissue and cell lines derived from human prostate tumours were low or undetectable.

PEDF, already known to be anti-angiogenic in the eye, was also shown to be so in prostate and pancreas cells, using the microvascular endothelial cell migration assay. Angiogenesis sustains tumour growth and metastasis; and in the prostate, androgens can stimulate angiogenesis through vascular endothelial growth factor, increasing prostate vascularity. Using a rat castration model, the effect of androgen ablation (currently part of current prostate cancer treatment) on PEDF levels was studied. PEDF levels rose significantly for three days post-castration compared with normals. But is this also seen in humans? Indeed, prostate biopsy specimens were studied before and after androgen ablation, and a similar rise in PEDF was seen.

To determine whether exogenous PEDF expression could suppress tumour growth, recombinant PEDF was added to cultured prostate tumour cells. PEDF levels rose, triggering apoptosis of both endothelial cells and epithelial cells. Under hypoxic conditions – mimicking the situation in growing tumours – this effect was enhanced further.

This work suggests that treatments involving replacing PEDF could prove useful in stabilizing or suppressing tumour growth in prostatic cancer patients.

8 Doll, J.A. *et al.* (2003) Pigment epithelia-derived factor regulates the vasculature and mass of the prostate and pancreas. *Nat. Med.* 9, 774–780

Miscellaneous

Subtle differences in SOD1 structure provide clues to ALS's destructive work

Researchers from the USA and UK have discovered subtle but deadly differences in the shape of protein structures that are mutated in familial amyotrophic lateral sclerosis (FALS), the inherited form of Lou Gehrig's disease [5].

ALS affects about one in 1000 people; 10% of cases are familial, 90% sporadic. The disease is an autosomal dominant neurodegenerative disorder characterized by the destruction of large motor neurones in the spinal cord and brain, and results in progressive paralysis, usually leading to death 2–5 years after onset of symptoms. Of the familial cases, 20% are associated with mutations in copper-zinc superoxide dismutase 1 (SOD1).

FALS SOD1-containing aggregates form in diseased motor neurones of FALS patients. It is widely thought that these aggregates kill motor neurones, leading to paralysis. Until now, however, the molecular basis underlying aggregate formation has been unknown.

P. John Hart, Professor of Biochemistry and Director of the University of Texas Health Science Center's X-ray Crystallography Core Laboratory (http://www.uthscsa.edu), and collaborators have used X-ray crystallography to study the structure of pathogenic SOD1 mutants defective for metal binding (a feature suggested to underlie the toxicity of many pathogenic SOD1 mutants). They showed that two mutants undergo conformation changes that facilitate their polymerization, through extensive native protein–protein interactions, into larger filamentous assemblies; normal SOD1 does not. This could be a toxic property common to mutants of SOD1 linked to FALS.

The impact of these results could go much further than ALS: neural damage mediated by toxic, aggregation-prone proteins is also seen in other neurodegenerative diseases, such as Alzheimer's, Parkinson's, Huntington's and prion disease. Hart's observations link several pathogenic SOD1 mutations to deleterious protein aggregation, making it possible to draw parallels between ALS and these diseases.

The next step is to develop compounds that will bind these mutant proteins, thus preventing aggregation.

5 Elam *et al.* (2003) Amyoid-like filaments and water-filled nanotubes formed by SOD1 mutant proteins linked to familial ALS. *Nat. Struct. Biol.* 10, 461–467

Chemical warfare from under the sea

Populations of marine plants and animals can be devastated by pathogenic microbes. However, many sessile organisms, such as seaweeds and sponges, suffer remarkably low levels of microbial infection, despite lacking cell-based immune systems. This is partly explained by a recent study showing that seaweeds can defend themselves from specific pathogens with naturally occurring antibiotics [6].

Disease resistance in seaweeds has been little studied and seaweed diseases are poorly understood, with the exception of those species that are commercially important (e.g. seaweed used in sushi). This study reports the isolation of a potent antifungal compound contained in the common seaweed species *Lobophora variegata,* which reveals an unusual chemical structure not seen before in plants. Using bioassay-guided fractionation, the group isolated and characterized a 22-membered cyclic lactone, lobophorolide [6], of presumed polyketide origin, with sub-μM activity against pathogenic and saprophytic marine fungi.

'We have discovered a new antibiotic with a complex chemical structure that structurally resembles two groups of macrolide antibiotics (i.e. those that kill fungi) – one found in marine sponges and the other in blue-green algae,' says lead author Julia Kubanek (Georgia Institute of Technology, Atlanta; http://www.gatech.edu/). Only tiny quantities of this new compound are available, however, it is hoped that these findings provide the possibility of biomedical applications for the newly discovered antifungal compound.

Scientists still need to determine whether or not the seaweed is the original source of the antibiotic. It could be the by-product of a symbiosis between the seaweed and an unidentified microbe. If so, it would be a rare example of such a chemical defense for plants and animals. However, these findings suggest that seaweeds use targeted antimicrobial chemical defense strategies and that secondary metabolites that are important in the ecological interactions between marine microbes and larger microorganisms could be a promising source of novel bioactive compounds.

6 Kubanek, J. *et al.* (2003) Seaweed resistance to microbial attack: a targeted chemical defense against marine fungi. *Proc. Natl. Acad. Sci. U. S. A.* 10.1073/pnas.1131855100 (http://www.pnas.org)

A detailed look at cholera pili

The molecular structure of a key cholera protein has been solved using two complementary techniques [7]. This structural knowledge will be helpful in the development of therapies and vaccines against cholera and related diseases.

Cholera is no longer a serious problem in developed countries, however, in areas of the world with poor sewerage systems, the disease is still a major threat to public health. Researchers headed by John Tainer at the Scripps Research Institute (http://www.scripps.edu/) have taken an important step towards developing a new vaccine or therapeutic against the disease.

Vibrio cholerae, the bacterium responsible for cholera, relies on hair-like surface structures known as pili for movement across the mucosal surfaces of the host. These surface proteins also enable the bacteria to clump together to evade an immune response. An agent that could compromise the structure or function of the pili would therefore be a good starting point for drug development. However, detailed structural information has been difficult to obtain.

Pili comprise thousands of copies of a pilin protein subunit stacked into a long, thin aggregation; X-ray crystallography alone would yield little information about such a flexible macroassembly. To get round the problem, the researchers from Scripps used the complementary techniques of X-ray crystallography and cryo-electron microscopy to look at the structure at different scales.

First, the crystal structure of an individual pilin was determined at 1.3 Å resolution. Although a fragment of the structure was missing, its configuration was inferred from the crystal structure of a related pilin. Next, to determine how pilins organize into pilus filaments, the team used cryo-electron microscopy, a technique well-suited to visualizing protein assemblies.

This analytical pincer movement enabled the team to build a more detailed model of pili than either technique alone could provide. Because the type IV pili characterized in this study are key virulence factors in many human pathogens, they represent a potentially general antibacterial target.

7 Craig, L. *et al.* (2003) Type IV pilin structure and assembly: X-ray and EM analyses of *Vibrio cholerae* toxin-coregulated pilus and *Pseudomonas aeruginosa* PAK pilin. *Mol. Cell* 11, 1139–1150

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