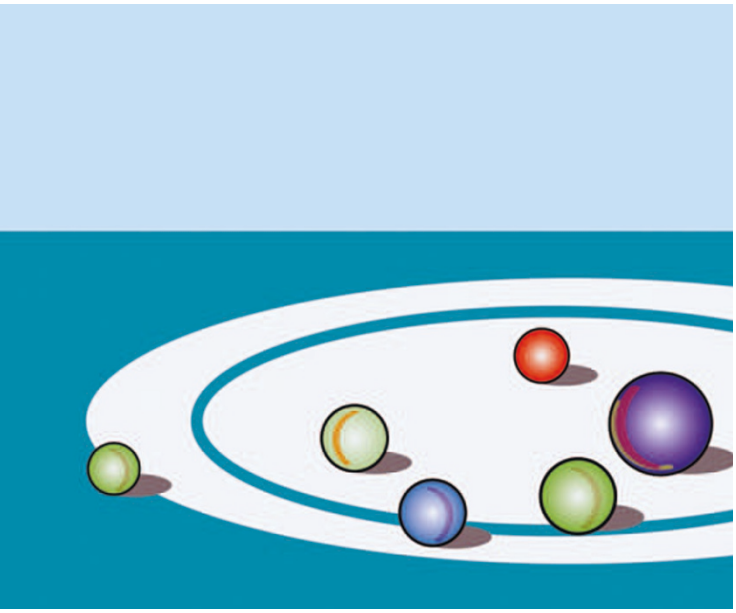
ANTIVIRAL DRUGS

Strategic targeting of the host



Current drugs against HIV type-1 (HIV-1) target a variety of viral proteins. Unfortunately, the genes encoding these proteins mutate rapidly, leading to drug-resistant viral variants arising during therapy and limiting the usefulness of a particular drug. An alternative approach is to inhibit non-essential host-cell proteins that are required for viral replication. In the May issue of *Nature Cell Biology*, Mark O'Connor and colleagues have identified a host protein, the ataxia-telangiectasia-mutated (ATM) kinase, as a new target for the development of antiretroviral therapies to treat HIV-1. This new research could allow the development of a new therapy that is effective against all HIV strains, substantially reducing the likelihood of drug resistance.

Mammalian cells respond to DNA damage by activating a number of response pathways to maintain genomic integrity. Integration into the host DNA is an essential step in the retroviral life cycle. A viral integrase is necessary for integration of viral product into the host genome, which cleaves the host DNA and then utilizes the host repair response in order to complete the integration process. Retroviral infections, such as those caused by HIV-1, activate a poorly understood repair pathway.

Evidence exists that the Ku-dependent non-homologous end-joining (NHEJ) pathway is required to support efficient retroviral infection. There is also some evidence of involvement of ATM kinase and a related protein, ataxia Rad-related (ATR), in this pathway. Both ATM and ATR are phosphatidyl-3-OH-kinase-like serine/threonine kinases that regulate cellular responses to DNA damage by controlling cell-cycle arrest and DNA-repair pathways. ATM is mutated in a rare genetic disease, A-T, that can lead to cancer, particularly leukaemia and lymphoma, and premature ageing.

The authors used genetic and pharmacological approaches to show that ATM has an important role in retroviral replication. They showed that the activity of HIV-1 integrase stimulates an ATM-dependent

DIABETES AND OBESITY

Battling the bulge with AGF

Creating a conventional knockout mouse can be a bit of a gamble — will there be a useful phenotype, or will it be embryonically lethal? Oike *et al.* have hit the jackpot in this respect by knocking out the gene encoding angiopoietin-related growth factor (AGF), which helped them establish that AGF, a circulating angiogenic orphan peptide secreted by the liver, counteracts obesity and related insulin resistance.

Although the AGF knockout proved to be 80% embryonically lethal due to cardiovascular defects, the surviving pups grew — too well. In fact, by 24 weeks of age, AGF-knockout mice (*Angptl6*$^{-/-}$) weighed approximately twice as much as wild-type mice. This increase was due to grossly enlarged adipocytes in their white adipose tissue and large amounts of lipid deposition in liver, skeletal muscle and brown adipose tissue. Conversely, mice transgenic for AGF were smaller than their wild-type counterparts, and managed to keep their shape even on a high-fat diet. Last but not least, wild-type mice with increased weight due to a high-fat diet lost significant amounts of weight when treated with an adenovirus encoding mouse AGF.

None of these effects could be attributed to differences in food intake. However, metabolic analysis of *Angptl6*$^{-/-}$ mice revealed a decrease in body temperature and whole-body oxygen consumption rates, indicating that lower energy expenditure due to reduced adaptive thermogenesis (heat production in response to diet or environment temperature) was the underlying cause for weight gain.

It was previously known that the brown adipose tissue and skeletal muscle regulate adaptive thermogenesis, mediated by peroxisome proliferator-activated receptor-α (PPARα), PPARδ, PPARγ and the PPARγ co-activator 1β (PGC-1β) and PGC-1α, in response to energy overload. All these factors were affected in the knockout mice. Furthermore, it was known that p38 mitogen-activated protein kinase (MAPK) enhances the stabilization and activation of the PGC-1 protein, and the present study established that AGF can activate p38 in muscle. The authors therefore propose that AGF stimulates fat burning in peripheral tissues through the p38 MAPK pathway and induces downstream effects on respiration and gene expression linked to mitochondrial uncoupling and energy expenditure. Furthermore, the angiogenic effects of AGF might antagonize obesity by facilitating energy expenditure through an increased number of microvessels, as observed in AGF transgenic mice.

Finally, the knockout mice were not only overweight, but were also affected by obesity-related severe hyperinsulinaemia, indicating insulin resistance. This condition, inducible by a high-fat diet in wild-type mice, could be reversed by adenoviral expression of AGF.

These studies clearly establish AGF as a potential target for developing pharmacological interventions to counteract obesity and related insulin resistance — the race will be on to identify the receptor for AGF and agonists that can mimic its effects.

Alexandra Flemming

References and links

ORIGINAL RESEARCH PAPER Oike, Y. *et al.* Angiopoeitin-related growth factor antagonizes obesity and insulin resistance. *Nature Med.* **11**, 400–408 (2005)

DNA-damage response and that a deficiency of ATM sensitizes cells to retrovirus-induced cell death. ATM helps to repair DNA damage caused by viral integration in the host-cell genome and is therefore essential to the survival of infected cells. Furthermore, treating HIV-1-infected cells with an ATM-specific small-molecule inhibitor, KU-55933 (not effective in targeting ATR), led to increased cell death and suppression of both wild-type and drug-resistant HIV-1 replication.

If effective in animal and clinical studies, ATM inhibitors could provide a new class of anti-HIV inhibitors. However, an important and outstanding question is whether or not inhibition of ATM will be associated with the same safety issues as the genetic disease. The effect of ATM kinase inhibition is likely to be less dramatic than the complete absence of ATM protein.

Melanie Brazil

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ORIGINAL RESEARCH PAPER Lau, A. *et al.* Suppression of HIV-1 infection by a small molecule inhibitor of the ATM kinase. *Nature Cell Biol.* **17** Apr 2005 (doi:10.1038/ncb1250)

GENE EXPRESSION

Do microarrays match up?

Disparities between microarray data from different groups working on similar samples has made many question the validity of this widely adopted technology. Although the 'minimal information about a microarray experiment' (MIAME) guidelines set standards for the publication of microarray data, they do not address experimental reproducibility. As gene-expression data rapidly accumulate in the public domain, three papers in *Nature Methods* provide a timely investigation into the reproducibility of microarray data and suggest that with appropriate caution such data can be used with confidence.

One of the main issues when comparing microarray data is consideration of the metrics generated by different technology platforms. There is a tremendous choice of platforms available and much diversity in protocols for sample preparation, imaging and analysis. Furthermore, whereas some groups report the absolute level of expression of a particular gene, others compare the relative transcription of genes. This makes meaningful comparisons of gene-expression data from different sources challenging.

The three papers investigate different aspects of microarray reproducibility. Larkin *et al.* directly compared the performance of two microarray platforms — an in-house-developed two-colour cDNA array and a commercial oligonucleotide array — in a study of the effects of chronic and acute exposure of angiotensin II on cardiac gene expression in mice. Irizarry *et al.* studied the impact of inter-laboratory variation by providing a consortium of ten laboratories with an identical RNA sample processed according to individual laboratory protocols, and then comparing the results obtained from three widely used microarray platforms. Finally, the Toxicogenomics Research Consortium (TRC) used in-house and commercial microarrays with identical RNA samples to assess the variability caused by sample handling, imaging and data analysis.

The studies show that results between platforms are remarkably consistent. Larkin *et al.* report that most genes had similar expression patterns but that the relative amplitude of expression was greater according to the commercial array. Some genes had divergent expression patterns between platforms, but principal-components analysis clustered these genes by experimental treatment rather than platform. Mapping probes from both arrays to the genome revealed that the two platforms interrogated different sequences for these divergent genes; Larkin *et al.* suggest that the presence of poorly or non-annotated splice variants might explain this inconsistency.

Considerable variation between laboratories using identical RNA samples was identified by both Irizarry *et al.* and the TRC study, although the TRC study showed that reproducibility improved markedly after standardizing protocols for RNA labelling, hybridization, array processing, data acquisition and normalization.

All three papers agree that using a standard procedure to normalize data relative to controls provides a more meaningful value and eliminates technical variability caused by probe and target molecules. Moreover, the TRC study showed that the use of gene-ontology nodes to analyse groups of genes in lieu of direct gene-by-gene comparison identified significant biological themes even with low levels of correlation between data from different platforms and laboratories.

Despite some disagreement, the authors reach a common consensus that standardization of experimental and analytical procedures is warranted. These studies should boost confidence that robust and reproducible results can be obtained using microarrays.

Joanna Owens

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ORIGINAL RESEARCH PAPERS Larkin, J. E. *et al.* Independence and reproducibility across microarray platforms. *Nature Meth.* **2**, 337–343 (2005) | Irizarry, R. A. *et al.* Multiple-laboratory comparison of microarray platforms. *Nature Meth.* **2**, 345–349 (2005) | Members of the Toxicogenomics Research Consortium. Standardizing global gene expression analysis between laboratories and across platforms. *Nature Meth.* **2**, 351–356 (2005)