Testing the information-theoretic approach

In collaboration with Murali Ramanathan at the University at Buffalo (http://www.buffalo.edu), the two Penn State scientists tested their approach by applying it to two previously published data sets:

- a set of 517 genes that were expressed after stimulation of serum with human fibroblasts [3]; and
- a set of 6108 cell-cycle regulated yeast genes [4].

Using visual inspection and statistical analysis, the investigators demonstrated that SOM plus KL divergence is superior to hierarchical clustering plus PC, or even to SOM plus PC [2]: the SOM algorithm was able to detect clusters with distinct patterns of temporal gene expression but the cluster plots obtained with KL divergence were better separated and more dense than those obtained with the PC distance measure. By contrast, hierarchical clustering produced a large amount of false positives, and many clusters contained only a single gene. Acharya and Kasturi are now working on improving their method (a more user-friendly version of the programme will be available soon on Kasturi's website (http://www.cse.psu.edu/~jkasturi).

More meaningful information

An important future direction would be to modify the approach to take into account slight phase shifts in transcript production, suggests Neumann. 'Genes can be controlled the same way as another gene, but they may take a little longer to get expressed,' he explains. 'You would supposedly see profiles that look very similar but have a bit of a time shift; people refer to this as a phase shift. Unless you explicitly take that into account in your analysis [and only a few papers have done so to date], all these tools are doing time-to-same-time comparisons; they don't address the whole problem.'

But the SOM plus KL divergence approach will already be an important addition to the array of tools that scientists can use for the analysis of gene expression data: 'If you use our method, you can come up with well separated clusters that probably won't be obtained by other methods,' concludes Acharya. He predicts that this could translate into more meaningful information about gene function – and could thus improve the drug discovery process.

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Spinach makes a safer anthrax vaccine

Hillary E. Sussman, freelance writer

The current form of the vaccine against anthrax infection has been licensed for human use since 1970 but has recently been characterized as sub-optimal. In response, a resurgence of research to produce a purer, safer vaccine has ensued. One such effort, by scientists at Thomas Jefferson University (http://www.tju.edu), has revealed that spinach can be used as a vehicle for the production of an edible vaccine against anthrax [1].

The anthrax bacterium

Anthrax is caused by the spore-forming bacterium *Bacillus anthracis*, which exists as spores in the soil and, therefore, commonly affects grazing animals such as cattle and sheep. Human infection, although rare, occurs following direct skin contact with infected animals or their wool, hides or tissues, by ingestion of contaminated meat, or via inhalation of the spores. Left untreated, inhalation anthrax is almost always fatal and early intervention with antibiotics, such as ciprofloxacin, is essential.

The status quo

Vaccination is recommended for persons at risk of exposure to anthrax spores. The current vaccine is based on cell-free culture supernatants of an attenuated strain of *B. anthracis* adsorbed on aluminium hydroxide (in the USA) or precipitated with aluminium phosphate (in the UK) – aluminium acts as an adjuvant. It is incompletely characterized and difficult to standardize and, therefore, exhibits inconsistency between lots. It is also relatively reactogenic, with side effects including a possible link to Gulf War Syndrome (whose symptoms include chronic fatigue, depression, skin rashes and gastrointestinal disorders [2]),

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and requires a lengthy dosing schedule, all of which suggest the need for an improved, alternative vaccine [3].

The main immunogenic component of the current vaccine has been determined to be the protective antigen (PA), and vaccination with PA alone can induce protective immunity to anthrax [4,5]. PA binds mammalian cell surface receptors, where it is proteolytically cleaved and activated to form a heptameric pore-like structure that binds either edema factor (EF) or lethal factor (LF) to form edema toxin and lethal toxin, respectively. Following endocytosis of the toxin complex, PA facilitates the passage of the toxins into the host cell cytoplasm where they disrupt normal signalling pathways leading to cell lysis, toxic shock and, ultimately, death [6,7]. However, 'if you can block the very first stage – binding of the PA to the receptor – you block the mechanism-of-action of the toxin and essentially block the disease,' says Alexander Karasev, Assistant Professor of Microbiology and Immunology at Thomas Jefferson University.

Plant powerhouses

'The new vaccines will be based on recombinant PA and will be much purer than the current vaccine,' says Meryl Nass, a Diplomat on the American Board of Internal Medicine (http://www.anthraxvaccine.org), but 'whether a pure PA vaccine will be safer is a big question.' Stephen Leppla, Senior Investigator in the Microbial Pathogenesis Section at the National Institute of Allergy and Infectious Diseases, NIH (http://www.niaid.nih.gov) concurs, 'The concerns about the existing vaccine may well apply to protective antigen-based vaccines regardless of whether they are made in plants, bacteria, yeast or other systems. There is no *a priori* reason to suggest that plant-derived vaccines will produce fewer side effects.'

Nevertheless, many scientists believe that it is unlikely that the PA protein itself is associated with adverse reactions. Furthermore, plant-based vaccines are appealing. 'One of the beauties of the plant system is that there are no pathogens that infect both plants and animals,' says Karasev, and without having to continuously screen the production medium for contaminants, screening costs are significantly reduced. Mohammad Azhar Aziz, Senior Research Fellow at the Centre For Biotechnology, Jawaharlal Nehru University, India (http://www.jnu.ac.in) adds that the 'production of subunit vaccines in plants offers the additional unique advantage of delivery in commonly consumed foodstuff, which may enhance the availability and ease of delivering immunizations.' Aziz is part of a group of scientists that successfully integrated the PA gene into the nuclear genome of tobacco plants last year [8].

Because there is some public opposition to the genetic modification of plants, and tobacco is considered an experimental plant, the scientists at Thomas Jefferson University designed a system to transiently express PA within a normal spinach plant (Fig. 1), moving yet another step closer to an edible anthrax vaccine. Specifically, a fragment of PA that represents most of the receptor-binding domain was expressed as a translational fusion with a capsid protein on the outer surface of tobacco mosaic virus, and spinach was inoculated with the recombinant virus particles. 'One of the rationales for using just a fragment of the protein is that basically you don't need the whole protein to elicit a protective immune response', Karasev says.

Figure 1. Spinach plants producing protective antigen (PA) of *Bacillus anthracis*. Figure kindly provided by Alexander Karasev (Thomas Jefferson University in Philadelphia, PA, USA; http://www.tju.edu).

The anthrax challenge

The plant-expressed PA is highly immunogenic in laboratory animals but producing antibodies that are specific to PA is not sufficient. 'The key question is whether or not the antibodies are protective,' points out Sanford Kimmel, Professor of Family Medicine at the Medical College of Ohio (http://www.mco.edu). The group plans to first test whether these antibodies inactivate the anthrax toxin *in vitro* and then determine whether they protect laboratory animals against anthrax. Karasev also admits that evoking a good systemic immune response after the vaccine is delivered through the digestive system is 'probably the biggest challenge today'. The PA fragment was extracted and purified from the spinach to test it as an immunogen 'but in theory if you solved the problem of eliciting a strong immune response you can probably just eat it as a vegetable salad,' remarks Karasev.

The development of plant-based vaccines to protect against many other diseases, such as HIV-1, hepatitis B, rabies and non-Hodgkin's lymphoma, are ongoing.

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Drug reactivates genes to inhibit cancer

Vida Foubister, freelance writer

Many tumour suppressor genes become inactivated in cancer cells, and a new drug has been found that appears to reverse this process in an animal model. Zebularine, which acts as an inhibitor of DNA methylation, significantly reduces the size of malignant tumours in mice [1]. Scientists at the University of Southern California/Norris Comprehensive Cancer Center (http://ccnt.hsc.usc.edu/) believe that zebularine holds promise as a therapeutic because it is the first DNA methylation inhibitor to reactivate silenced genes through oral administration.

DNA methylation inhibitors

Genes that have a role in the control of cell growth are often not expressed in cancer. Until recently, the field has focused on the mutation or loss of these genes. However, of late there has been considerable attention directed at the silencing of tumour suppressor genes, which can occur as a result of abnormal methylation at their promoter region [2].

'You have a perfectly good gene there and it becomes silent,' said Peter A. Jones, Director of the USC/Norris Comprehensive Cancer Center. 'This then gives you a therapeutic target because if you can reactivate the gene by reversing its methylation, you can turn it back on again.' Another silencing mechanism that has also come to the fore involves changes to the chromatin structure in the promoter region of tumour suppressor genes. These changes are being targeted clinically through the use of histone deacetylase inhibitors, including valproic acid and suberoylanilide hydroxamic acid (SAHA).

The focus on methylation has reawakened interest in two drugs, 5-azacytidine and 5-aza-2′-deoxycytidine, which initially went through cancer clinical trials without impressive outcomes. At the time, their function as DNA methyltransferase inhibitors was unknown and, therefore, the studies were not appropriately designed to inhibit methylation.

'These drugs have come back into the clinic and have very interesting and promising early results,' said Jean-Pierre Issa, Associate Professor in the Department of Leukemia at The University of Texas MD Anderson Cancer Center (http://www.mdanderson.org/). 'This, and the basic science rationale that if you could strip off the DNA methylation the gene underneath is perfectly fine, has prompted people in companies to start looking for other inhibitors of DNA methylation.'

Based on a recently completed Phase III trial, Pharmion Corp. (http://www.pharmion.com/) plans to file a new drug application with the US Food and Drug Administration (FDA; http://www.fda.gov) this year for 5-azacytidine, commercially referred to as azacitidine, as a treatment for patients with myelodysplastic syndromes. SuperGen (http://www.supergen.com) has begun Phase III clinical trials with 5-aza-2′-deoxycytidine or decitabine, a drug that Issa currently works with, for advanced myelodysplastic syndrome.

A fortuitous discovery

Despite the promise of azacitidine and decitabine, these compounds must be administered intravenously or subcutaneously. Thus, a search is under way for chemically stable analogues of these drugs that retain the ability to inhibit DNA methylation.

Zebularine – 1-(β-D-ribofuranosyl)-1,2-dihydropyrimidin-2-one – was not

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