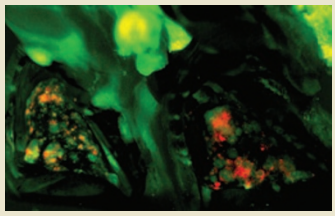
pH-activated cancer probes



Fluorescent probes that selectively label viable tumor cells with minimal background signal from healthy tissue could provide a valuable aid to resection surgery and the monitoring of cancer therapy. Exploiting the acidic microenvironment of lysosomes to differentiate between the extracellular and intracellular milieu, Urano *et al.* design pH-sensitive boron dipyrromethene–based probes. They target these probes to cancer cells by conjugating them to an antibody specific for the human epidermal growth factor receptor 2 (HER2). Not only does the pH sensitivity of the probe mean that living cells are preferentially visualized but also specificity for HER2 increases target-to-background signal ratio. As is evident from the image on the right, a pH-activated probe distinguishes HER2-overexpressing murine lung metastases (green) from HER2-deficient tumors expressing red fluorescent protein (red) far better than a constitutively active control probe (left). The latter generates green fluorescence not only from HER2-overexpressing tumors but also from healthy tissue and HER2-deficient tumors (yellow). The principle used in the study should be relevant to any cell surface–targeting probe that is internalized through lysosomes. (*Nat. Med.*, advance online publication, doi: 10.1038/nm.1854, 7 December 2008) *PH*

Tracking key cancer proteins

A conundrum posed by cancer therapies is how to predict the seemingly individualized responses to many drugs currently in use. By surveying the levels of over a thousand proteins in single cells of a human lung carcinoma line treated with the cancer drug, camptothecin, Alon and colleagues show that the fate of a cell to die or to grow correlates with marked changes in only a few proteins. Using existing 'CD tagging' technology, in which a retrovirus inserts a fluorescent label (enhanced yellow fluorescent protein) into the intron of a protein-encoding gene, the researchers tagged 1,260 different cellular proteins. They then used quantitative fluorescent microscopy to assess both protein levels and subcellular locations for the 48 hours after drug treatment. Patterns emerged in the amounts and types of proteins produced in response to camptothecin treatment; levels of camptothecin's target protein, topoisomerase I, almost immediately decreased, whereas levels of other proteins changed more slowly. Most proteins whose level changed did so in the majority of cells, but 24 showed a bimodal distribution (that is, in some cells the levels went up, whereas in others, it went down). The amounts of two bimodally expressed proteins, RNA helicase and replication factor RFC1, correlated with survival; when levels rose, the cell survived, but when it fell, the cell died. The approach provides a dynamic means of pinpointing proteins responsible for cancer cell survival. (*Science* **322**, 1511–1516, 2008) *LD*

De novo bacterial sequencing

Next-generation sequencing is now showing its potential as an alternative to shotgun Sanger sequencing, long the workhorse of bacterial genome discovery. Reinhardt *et al.* report the use of cyclic array sequencing technologies from Illumina (San Diego) or Roche (454 Life Sciences; Basel) to assemble a eubacterial genome from short DNA fragment reads without the use of a reference genome—a process referred to as *de novo* assembly. In contrast to the technology in the first report of *de novo* assembly last year, the technology described in the current paper requires about ten times less raw data, and is especially useful for sequencing the rapidly evolving genomes of bacteria—which, from even closely related strains, may differ by many genes often associated with pathogenesis. The authors use their method to sequence the genome of the plant pathogen *Pseudomonas syringae,* a relative of the human pathogen *Pseudomonas aeroginosa*, which accounts for ~10% of all infections acquired in United States' hospitals. They then identify unique *P. syringae* candidate virulence genes that would have been missed by existing assembly methods requiring a reference genome. The technique opens the door for the large-scale sequencing of many individual bacterial isolates, a capability essential for understanding mechanisms of bacterial evolution and toxicity. (*Genome Res.*, published online, doi:10.1101/gr.083311.108, 17 November 2008) *CM*

Inside-out antivirals

Phosphatidylserine is actively maintained in the inner leaflet of the membranes of healthy cells, but 'flips' and becomes exposed on the cell's exterior during apoptosis. Having first shown that this phenomenon is triggered by infection with a range of viruses *in vitro*, Soares *et al.* exploit the loss of asymmetry in the membranes of virally infected cells and enveloped virions by demonstrating the potential of aminophospholipids as antiviral targets. Inspired by studies suggesting the immunosuppressive and anti-inflammatory potential of exposed phosphatidylserine, they show that a chimeric antibody (bavituximab) against complexes comprising a phosphatidylserine-binding plasma protein and anionic phospholipids enhances the robustness of the immune response to viral infection. Treatment with bavituximab not only cured guinea pigs lethally infected with Pichinde virus (a model for Lassa fever virus, a potential bioterrorism agent), but also rescued mice with lethal cytomegalovirus infections. The antiviral effects can primarily be attributed to direct virus clearance from the bloodstream and antibody-dependent cellular cytotoxicity. Bavituximab treatment appears to be well tolerated and augments the antiviral effect of ribavirin, the drug of choice for treating Lassa fever. (*Nat. Med.* **14**, 1357–1362) *PH*

Modeling neurological disease

An important and relatively near-term application of pluripotent cells is *in vitro* disease modeling. Unlike primary cells from patients, pluripotent cells could provide an unlimited supply of cells for drug screening and for investigation of pathological mechanisms. Three recent reports use such an approach to model motor-neuron disease. Motor neurons cannot be isolated from humans. To study the contribution of astroctyes to the death of motor neurons in amyotrophic lateral sclerosis (ALS) using human cells, Marchetto *et al.* and Di Giorgio *et al.* co-cultured motor neurons derived from human embryonic stem cells and astrocytes expressing ALS-associated mutations in superoxide dismutase 1 (SOD1). They found that SOD1-mutated astrocytes are toxic to motor neurons but not to other neural cells and gained insight into disease mechanisms mediated by reactive oxygen species and pro-inflammatory factors. Drug testing identified compounds with a protective effect on motor neurons. In a separate study using induced pluripotent stem cells rather than embryonic stem cells, Ebert *et al.* reprogrammed fibroblasts from a boy with spinal muscular atrophy and showed that motor neurons differentiated from the pluripotent cells recapitulate some features of the disease phenotype. (*Cell Stem Cell* **3**, 649–657, 2008; *Cell Stem Cell* **3**, 637–648, 2008; *Nature*, advance online publication, doi:10.1038/nature07677, 21 December 2008) *KA*

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