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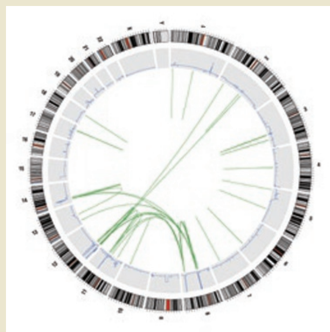
Paired-end sequencing of oncogenic changes



Campbell *et al.* use massively parallel sequencing to efficiently and comprehensively identify genes and other genomic regions affected by somatic genetic changes associated with cancer. Their analysis of the ~32 nucleotides closest to the ends of short DNA fragments derived from two lung cancer cell lines identifies hundreds of rearrangements, insertions, deletions and copy number variants at single base pair (bp) resolution. Mapping the ends of genomic fragments <500-bp long onto a reference genome enables detection of rearrangements within and between chromosomes (depicted), as well as insertions and deletions. Scoring the frequency with which sequences are detected can reveal variations in genomic copy number. It is suggested that sequencing to a greater depth than used in the study might also reveal point mutations associated with malignancy. Well-defined cancer-associated mutation patterns deduced from application of the paired-end sequencing strategy to more samples and distinct tumor types should be invaluable in understanding the causes of cancer and developing appropriate therapies. (*Nat. Genet.*, advance online publication, doi: 10.1038/ng.128, 27 April 2008) *PH*

Tackling Alzheimer's via the endosome

Understanding the role of endocytosis in Alzheimer's may lead to a new means of potentiating treatments. In Alzheimer's pathogenesis, amyloid-β peptide (Aβ) is released from the membrane by sequential cleavage of membrane-bound β-amyloid precursor protein (APP) by the β- and γ-secretases. The β-secretase catalyzes the rate-limiting step and is most likely a better therapeutic target than the multifunctional γ-secretase. Because endocytosis of APP and β-secretase is required for Aβ release, Rajendran *et al.* set out to test the effectiveness of a membrane-tethered soluble β-secretase inhibitor. They found that the sterol-coupled peptide is transported to endosomes and effectively inhibits β-secretase cleavage of APP and Aβ production, whereas the free control is absent in endosomes and almost completely inactive. The sterol-coupled inhibitor is also enriched in cholesterol-sphingolipid membrane domains, sites in which APP cleavage preferentially occurs. Indeed, inhibitor variants linked to lipids with less affinity for these membrane microdomains (lipid rafts) were less potent than the sterol-coupled peptide. The sterol-linked inhibitor is also effective in *Drosophila* and mouse models of the disease. The authors argue that this membrane-tethering strategy might also be useful to design inhibitors against other disease-associated membrane proteins. (*Science* **320**, 520–523, 2008) *JWT*

Written by Kathy Ascheim, Peter Hare & Jan-Willem Theunissen

IVIG without plasma

The anti-inflammatory effects of intravenous immunoglobulin (IVIG) have been reproduced in mice using a recombinant form of the Fc fragment of IgG, according to a new study by Ravetch and colleagues. IVIG—intravenous delivery of immunoglobulin purified from pooled human plasma—is effective against a range of autoimmune diseases, but the molecular basis of this therapy is not fully understood. Building on previous work pointing to the importance of the sialic acid residues on the glycan of the IVIG Fc, the authors investigated which of two possible sialic-acid linkages—an α2,3 or α2,6 linkage—promotes anti-inflammatory activity. Mass spectrometry analysis of IVIG Fc glycans showed a preponderance of the α2,6 linkage. The authors then generated preparations of IVIG Fc enriched tenfold in α2,6 sialic-acid linkages and found that they suppressed inflammation in mice at tenfold lower doses compared with IVIG Fc. A recombinant α2,6-sialylated IgG Fc, produced to control for possible contaminants in these plasma-derived preparations, had similar activity. These results illuminate the mechanism of IVIG's anti-inflammatory action and may lead to a more potent therapeutic. (*Science* **320**, 373–376, 2008) *KA*

Self-renewal of mouse ES cells

Culture media for maintaining mouse embryonic stem (mES) cells in a pluripotent state contain any number of components that activate signaling pathways, such as feeder cells, cytokines, growth factors and serum. In particular, they usually include leukemia inhibitory factor (LIF), which activates the STAT3 pathway, and bone morphogenetic protein (BMP) or serum, which activate inhibitor-of-differentiation proteins. But is activation of signaling pathways truly required to derive and culture mES cells, or is it sufficient to block pathways that promote differentiation? Smith and colleagues report that LIF and BMP or serum are in fact dispensable if the culture medium contains inhibitors of differentiation-inducing signaling through fibroblast growth factor receptor tyrosine kinases, the extracellular signal–regulated kinase cascade and glycogen synthase kinase 3. The authors confirm that LIF is unnecessary by generating ES cells from *Stat3* homozygous knockout mice. They conclude that m ES cells have "an innate programme for self-replication," a finding that may aid the development of defined media for the culture of human ES cells. (*Nature*, **453**, 519–524, 2008) *KA*

Mutated human kinome collection

Varjosalo *et al.* describe a collection of 568 full-length protein kinase cDNAs (representing >93% of all human protein kinases) augmented by 425 cDNAs encoding catalytically inactive point mutants of 351 of these kinases. Kinase activity-deficient variants potentially reveal nonenzymatic roles of a kinase of interest. By screening this unique resource in a cell-culture model of Hedgehog signaling, the authors were able to uncover the roles of the DYRK2 and MAP3K10 kinases, acting through GLI transcription factors, in regulating this cancer-associated pathway. The genome-wide collection of protein kinases and their nonfunctional variants also enabled identification of a novel kinase required for reactivation of Kaposi's sarcoma herpes virus and previously described effectors of transforming growth factor β signaling. The findings, obtained using three different signaling models, underscore the potential of cDNA expression screening in systematically elucidating signaling intermediates and dissecting crosstalk between kinase pathways. The approach should complement loss-of-function strategies, such as those involving RNA interference. (*Cell* **133**, 537–548, 2008) *PH*