

Research Highlights

In this section we feature short articles on significant research publications from the Institute this year, in a style accessible to a non-specialist science reader.

Magnetic resonance imaging of pH *in vivo* using hyperpolarized ¹³C-labelled bicarbonate. Gallagher FA, Kettunen MI, Day SE, Hu D-E, Ardenkjær-Larsen JH, Zandt R, Jensen PR, Karlsson M, Golman K, Lerche MH, Brindle KM. *Nature* 2008; 453:940-3

Tumours have a lower pH, or are more acidic, than the surrounding tissue because the body's acid-base balancing system is disturbed. Spotting differences in acidity could be the key to finding small tumours and also for determining whether they are responding to treatment. However pH is difficult to measure in the human body. This paper describes a technique that dramatically increases the sensitivity of magnetic resonance imaging (MRI) by more than 10,000 times and which utilises the body's own pH balancing system to detect regions of low pH. Bicarbonate – which is more commonly known as baking soda – occurs naturally in the body and buffers changes in pH by inter-conversion with carbon dioxide (CO₂). Importantly, if the amount of bicarbonate and CO₂ could be measured, then the pH of the tissue can be calculated; however, this has never been possible in humans before. Using a form of bicarbonate that was tagged with a special type of non-radioactive carbon – called carbon 13 – the authors used this very sensitive MRI technique to measure tissue pH in mice, finding that they could distinguish tumour from normal tissue by differences in the pH. This technique is a potentially safe and non-toxic way of detecting tumours in humans, which could be used to detect tumours earlier and to determine the best treatment to give patients.

Regulation of *ERBB2* by oestrogen receptor-PAX2 determines response to tamoxifen. Hurtado A, Holmes KA, Geistlinger TR, Hutcheson IR, Nicholson RI, Brown M, Jiang J, Howat WJ, Ali S, Carroll JS. *Nature* 2008; 456:663-6

This paper addresses the mechanisms of action of the anticancer drug tamoxifen and the potential ways that tamoxifen resistance develops. It has been known for a long time that the gene *ERBB2* is the key feature of a certain subtype of breast cancer. This paper now shows that for tamoxifen to work in a different subtype of breast cancer (those caused by the estrogen receptor), it needs to prevent *ERBB2* from being switched on. This tamoxifen repression of *ERBB2* appears to use a control switch that is hidden within

the *ERBB2* gene itself. Tamoxifen works when the switch is held off, but a breakdown in this control mechanism can cause *ERBB2* to get turned on and this negatively influences the ability of tamoxifen to work. The authors found that a new regulator called Pax2 is implicated in this process which is a novel way for tumours to develop tamoxifen resistance.

Pro-neural transcription factors as cancer markers. Vias M, Massie CE, East P, Scott H, Warren A, Zhou Z, Nikitin AY, Neal DE, Mills IG. *BMC Medical Genomics* 2008; 1:17

Finding markers that can either accurately identify tumours early in their development, or that identify those cancers which are at increased risk of progressing, is important for improving treatment success and survival. As some tumours progress, there is an increase in the expression of some of those transcription factors that are normally switched on in the developing organs of embryos. Transcription factors which lead to a neuroendocrine phenotype are expressed in some prostate cancers and this paper describes pro-neural transcription factors that act as markers for aggressive phenotypes of prostate cancer. The markers belong to the basic helix-loop-helix (bHLH) family of proteins and normally regulate the development and maintenance of neural tissues. Working with a mouse model of prostate cancer and a prostate cancer cell line, the authors found that the pro-neural transcripts Hes6 and Ascl1, normally regulated in the brain, are up-regulated in high-risk cancers, which results in them assuming the gene expression characteristics of neurons as they develop and become more aggressive. In human tumour samples, the authors could discriminate between metastatic and primary tumours, and benign tissue according to levels of the pro-neural transcription factors Hes6 and Ascl1. The implication of these findings is that impairing the activation of pro-neural transcription factors could impact on cancer treatment by influencing cell fate and improving survival.

Species-specific transcription in mice carrying human chromosome 21. Wilson MD, Barbosa-Morais NL, Schmidt D, Conboy CM, Vanes L, Tybulewicz VL, Fisher EM, Tavaré S, Odom DT. *Science* 2008; 322:434-8

Sets of tissue-specific transcription factors establish each cell's gene expression during development and maintain it during adulthood by binding to DNA in a sequence-specific manner. Transcription factors often change the genes they potentially target, sometimes dramatically, in cancer and over evolution – the reason for which is poorly understood. Numerous causes have been suggested to contribute to these changes in transcription factor binding, including chromatin state, cellular environment, surrounding regulatory sequences, even diet and nutrition state.

To determine globally whether interspecies differences in transcriptional regulation are primarily directed by human genetic sequence or mouse nuclear environment, we used hepatocytes from an aneuploid mouse strain carrying human chromosome 21. This mouse strain carries DNA from two species in a single, perfectly uniform cellular environment, and thus all variables except genetic sequence are removed from the equation of transcription factor binding. We found that virtually all transcription factor binding locations, landmarks of transcription initiation, and the resulting gene expression observed in human hepatocytes were recapitulated across the entire human chromosome 21 in the mouse hepatocyte nucleus. Thus, in homologous tissues, genetic sequence is largely responsible for directing transcriptional programs; interspecies differences in epigenetic machinery, cellular environment, and transcription factors themselves play secondary roles.

Polygenes, risk prediction, and targeted prevention of breast cancer. Pharoah PD, Antoniou AC, Easton DF, Ponder BAJ. *New England Journal of Medicine* 2008; 358:2796-803

This paper examines whether recent findings that the greater part of breast cancer susceptibility may be caused by the combined effects of several low to moderate risk alleles, which could impact on public health policy and screening programmes. The authors examined seven low to moderate risk alleles – assuming that they interact multiplicatively, they determined that the lifetime risk of breast cancer for a woman with two copies of the low risk allele of each gene is 4.2%, whereas the lifetime risk for a woman with two copies of the high risk alleles of each gene is 23% (versus a population average of 9.4%).

Recent advances in screening technology mean that it could soon be possible to genotype every woman and generate a risk profile for all the known alleles that increase risk of breast cancer. The authors conclude that, making reasonable assumptions about the number of such alleles that might be identified, this could be a useful tool to categorise women according to their level of risk, which in turn would influence when they start regular screening. For example, a woman with all low risk variants of the alleles would enter a screening programme much later than a woman with all the high risk variants.

Statistical issues in the analysis of Illumina data. Dunning MJ, Barbosa-Morais NL, Lynch AG, Tavaré S, Ritchie ME. *BMC Bioinformatics* 2008; 9:85

In recent years new technologies have greatly increased the power and scope of genomics studies. One such technology is the Illumina BeadArray which is used in a

wide range of applications to analyse the characteristics of DNA sequences. Such arrays can consist of millions of randomly positioned beads, each three microns in diameter. A specific oligonucleotide probe is assigned to each bead type, which is replicated about 30 times on an array. The BeadArray is probed with the fluorescently tagged sequences of interest which hybridise to the beads. This paper focuses on the techniques used to analyse the pictures taken of the hybridised beads. One technique takes into account the variation in the bead data – for example, for a particular bead type, there might be 21 'hits' on one array, five on another, 53 on another. Taking into account the number of copies of a bead-type on each array is shown to increase the quality and the flexibility of the data analysis. The authors also warn against treating the 'negative control' bead types on an array as a 'base-line' against which the intensity of the bead colours is measured. This is shown to be detrimental. They also found that taking logs of data points, before summarising the data, substantially improved data quality. These techniques essentially enhance the differences between data points, making it easier to spot outliers and subtle details. This ultimately improves the power and flexibility of the analysis, and gives fewer false positive results.

The vitamin D receptor is a Wnt effector that controls hair follicle differentiation and specifies tumor type in adult epidermis. Palmer HG, Anjos-Afonso F, Carmeliet G, Takeda H, Watt FM. *PLoS ONE* 2008; 3:e1483

The Wnt signalling pathway plays an important role in the differentiation of cells in the epidermis, and can also influence tumour development in the skin. A key molecule in the pathway is β -catenin, which switches on many epidermal genes. Many of these genes also contain elements that respond to vitamin D and which are also induced independently of TCF, a transcription factor that β -catenin binds to. The vitamin D receptor is needed for hair follicle development induced by β -catenin. Analogues of vitamin D, such as the molecule EBI089, work with β -catenin to stimulate hair differentiation. Mistakes in Wnt signalling can lead to a range of skin tumours. Using transgenic mice, the authors found that hair follicle tumours (trichofolliculomas) are characterised by high levels of β -catenin and VDR. On the other hand, basal cell carcinomas were characterised by high β -catenin but low VDR levels. When they gave the mice EBI089, they could prevent β -catenin induced trichofolliculomas forming. Knocking out the VDR caused β -catenin to induce basal cell carcinoma. They conclude that the VDR switches on the target genes of the Wnt pathway independently of TCF and that vitamin D analogues could be developed into therapies in tumours which have been caused by inappropriate activation of Wnt signalling.