What it might take to unravel the 'lean mean machine' that is cancer

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Scientists from Sydney's Garvan Institute of Medical Research have published a paper, online today in *Nature Cell Biology*, describing gene expression in a prostate cancer cell: more sweeping, more targeted and more complex than we could ever have imagined, even five years ago.

The study shows that changes within the prostate cancer cell 'epigenome' (biochemical processes that target DNA and affect gene expression) alter the expression of many genes, silencing their expression within large regions of DNA - nearly 3% of the cell's genome.

Epigenetic 'events' include 'DNA methylation' and 'chromatin modification'. Methylation occurs when a methyl group - one carbon atom and three hydrogen atoms - attaches to a gene, determining the extent to which it is 'switched on' or 'switched off'. Chromatin, responsible for the physical coiling or structuring of DNA, can determine whether or not a gene is accessible for interaction with other molecules inside a cell.

Project leader Professor Susan Clark describes the typical cancer cell as a 'lean mean machine'. "Epigenetic changes reduce the available genome to a point where only the genes that promote cell proliferation are accessible in the cancer cell," she said.

"We can see that the epigenome is remodelled in a very consistent and precise way, effectively swamping the expression of any gene that goes against the cancer cell's interests."

"The swamping encompasses tumour suppressor genes, and all the neighbouring genes around them, as well as non-coding RNA, intergenic regions and microRNAs. Only those genes essential for growth activation are allowed to be active, while all the genes and regions that apply brakes are inactivated."

"We now have an epigenomic map of the prostate cancer cell - which we didn't have before. That has taken three years to develop, including the technology and methods to interpret our tissue samples."

"The map tells us that the tumour cell is very different from the healthy cell. It also tells us that it works in a programmed rather than a random way, and that it targets a significant part of the genome, rather than just single genes."

"It tells us that treating cancer will be far more complex than we imagined, as it will first involve understanding and reversing epigenetic change."

The findings are timely in that they coincide with very recent events and publications that have brought the concepts of the 'epigenome' and 'epigenetics' into world focus. In January 2010 the International Human Epigenome Consortium (IHEC) was launched in Paris (with Professor Clark on the interim steering Committee). Time magazine ran a feature on epigenetics in January, and Nature published two articles on the subject this month: one addressing the importance of IHEC and the urgency of pooling international mind power and resources; the other describing the infinite complexity of the project - orders of magnitude more challenging than the Human Genome Project.

The ultimate aim of IHEC is to produce a map of the human epigenome. The initial intention is to map 1,000 epigenomes within a decade. This will provide a healthy tissue base against which to compare the

epigenomes of diseased tissue.

The Human Genome Project, completed in March 2000, found that the human genome contains around 25,000 genes. It took 3 billion US dollars to map them.

We do not yet know how many variations the human epigenome is likely to contain - certainly millions - as a single person could have many epigenomes in a lifetime, or even in a day. The technological advances and computational power necessary to map the epigenome, therefore, remain incalculable.

The project at Garvan involved an initial bioinformatics phase; a comparative tissue analysis phase; and a data analysis phase.

The bioinformatics phase analysed publicly available microarray datasets (glass slides containing fragments of every gene across the genome) that had been done on prostate cancer.

Dr Warren Kaplan, Bioinformatics Analyst at Garvan's Peter Wills Bioinformatics Centre, developed new techniques to analyse the microarray data. "We designed a computer program which used a 'sliding window' - a window that computationally moves along the genome, noting the number of genes inside that window and how many of them are downregulated," he said.

"Some of the microarrays we used only measured mRNA - or the level of gene expression. Others measured the overall methylation status of the genes in that same region. It was an opportunity for us to examine the genome in a multi-layered way."

Once Kaplan had provided an initial map, Drs Marcel Coolen and Clare Stirzaker and Jenny Song from Professor Clark's lab found a way to treat

and analyse prostate cancer cells, allowing their comparative DNA methylation and chromatin states analysis against the microarray data.

Bioinformaticians within the Clark lab, Aaron Statham and Dr Mark Robinson, then developed novel methodologies to interpret resulting data - essentially tens of millions of numbers. "It was like cracking a code," said Aaron. "At first the data made no sense."

Professor Clark emphasises the importance of developing the new genome technology and knowhow that allows analysis of epigenetic processes.

"There is so much we still don't know," she said. "Already we have an idea of the complexity and how it might impact on the specific drug combinations that you will have to use to reactivate genes, non-coding RNAs and microRNAs within these cancer-affected regions."

"Now that we have a prostate cancer epigenome map, our next step will be to understand the mechanism that's driving the chromatin reduction, or genome reduction within these 'lean mean machines'. In other words, what's the link between the genetics and the epigenetics?"

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