Review

UHN Microarray Centre

Taking a closer look at aberrant methylation in cancer

Review of Feature Article: **Weber M, Davies JJ, Wittig D, Oakeley EJ, Haase M, Lam WL, Schubeler D.** Chromosome-wide and promoter-specific analyses identify sites of differential DNA methylation in normal and transformed human cells. Nature Genetics, 2005, 37(8):853

DNA methylation plays a crucial role in the control of gene activity and nuclear architecture[1]. Alterations in DNA methylation, such as hypermethylation at certain CpG-rich promoters and hypomethylation at repeated DNA sequences, are often found in cancers[2-4]. It is thought that alterations in DNA methylation may contribute to carcinogenesis by inactivating tumour-suppressor genes[4]. In this study, chromosomal and promoter-specific methylation profiles allowed Weber *et al.* to approximate the extent and localisation of differential methylation between primary and transformed cells[5]. The significance of the study is two-fold; it was the first to report the use of methylated DNA immunoprecipitation (MeDIP) for identifying changes in DNA methylation in transformed cells and the results of this study suggest that aberrant methylation of CpG island promoters in malignancy may be less frequent than previously hypothesised[5].

MeDIP is a powerful epigenomic technique that allows for the enrichment of methylated DNA using antibodies specific for methylated cytosine to immunocapture methylated genomic fragments. However, the need for whole genome amplification after immunopreciptation can introduce PCR biases, a consideration to keep in mind when using the MeDIP technique[1]. Another study using MeDIP by Zhang *et al*. combined MeDIP and tiling arrays to provide an almost complete DNA methylome of *Arabidopsis*[6].

By combining MeDIP with CpG island microarray (Human 12k CpG array from UHN Microarray Centre) hybridisation, Weber *et al*. created methylation profiles of all human chromosomes and assessed the frequency of aberrant CpG island methylation in transformed cells. A CpG methylation profile was generated for SW48 colon cancer cells and compared to methylation profiles of primary fibroblast and normal colon mucosa. This analysis showed that methylation levels of most CpG islands were maintained in the SW48 cancer cell line[5]. When comparing SW48 cells with normal colon mucosa, Weber *et al*. identified an almost identical population of unique sequences, suggesting that this methylation was linked to the transformed state. This study also found that the global distribution of methylated cytosine in SW48 cells was similar to that of primary fibroblasts. And, much like the normal fibroblast cells, the highest levels of methylation in the transformed cells occurred at gene-rich chromosomal regions. Several regions of marked hypomethylation were found in transformed cells, however, these were almost entirely in gene-poor regions.

Recently, other studies have been published that attempt to quantify the extent of DNA methylation at CpG island promoters and repeated DNA sequences in cancers[2,3]. A study by Nishiyama *et al.* provides the first report of a DNA sequence (*NBL2*) that can be either extensively hypermethylated or hypomethylated in cancer[2]. This study suggests that tumourigenesis-linked DNA methylation changes are much more flexible than commonly realised. Nishiyama *et al*. hypothesise that CpG island promoters that become hypermethylated in earlier stages of cancer may subsequently undergo demethylation during waves of tumour progression-linked DNA demethylation. In addition, a study by Lujambio *et al*. concluded that DNA hypermethylation contributes to the downregulation of microRNAs in human tumours[7].

The study by Weber *et al.* concludes that the combination of MeDIP with CpG island microarray hybridisation allows for the identification of epigenetically silenced genes in cancer cells and provides the first epigenomic map of DNA methylation in the human genome[5]. The results of this study found that the global pattern of CpG island methylation is

conserved between primary and transformed cells and that the number of hypermethylated CpG island promoters in transformed cells was unexpectedly low. A more comprehensive analysis including non-CpG island promoters may be required to conclusively determine whether or not preferential localisation of aberrantly methylated CpG island promoters occurs in chromosomal regions with differential methylation. Additional studies should reveal further insights into the dynamics and hierarchy of epigenetic regulation during normal development and disease[5].

Jonathan J. Davies (second author) will be presenting his research at the 1st Annual Toronto Functional Genomics Symposium, June 18-19, 2007

References

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