人全基因组甲基化芯片

表观遗传学

表观遗传学是与遗传学(genetic)相对应的概念。遗传学是指基于基因序列改变所致基因表达水平变化，如基因突变、基因杂合丢失和微卫星不稳定等；而表观遗传学则是指基于非基因序列改变所致基因表达水平变化，如 DNA 甲基化和染色质构象变化等；表观基因组学则是在基因组水平上对表观遗传学改变的研究。(epigenomics)

表观遗传的现象很多，已知的有DNA甲基化（DNA methylation），基因组印记（genomic impriting），母体效应（maternal effects），基因沉默（gene silencing），核仁显性，休眠转座子激活和RNA编辑（RNA editing）等。目前，国际上表观遗传学已经构成了系统遗传学研究的一个重要方面。

illumina DNA 甲基化芯片

DNA 甲基化对生命过程非常重要，是表观遗传学的重要调控方式，也是基因精确调控的方法之一，通过对位于基因启动子及第一外显子区的 CpG 岛的甲基化而抑制基因的表达。它在细胞正常发育、基因表达模式以及基因组稳定性中起着至关重要的作用。

甲基化逆转

亚硫氢酸盐处理基因组 DNA 可引起未甲基化的胞嘧啶 5’碳原子脱氨而最终变为尿嘧啶，而甲基化的胞嘧啶则对亚硫氢酸盐处理有较高的抗性。

1.准备 gDNA 500ng。

2.亚硫氰酸盐处理 gDNA，使 gDNA 中 5’碳原子没有被甲基化的胞嘧啶经逆转变为尿嘧啶。

3.将 gDNA 利用 NaOH 变性。

4.基因组全扩。

5.随机内切酶酶切，将基因组全扩结果切成大小均一的 DNA 片段。

6.杂交：和芯片进行杂交，芯片的微珠上连接有位点特异性探针，gDNA 酶切后产物与探针互补序列结合，完成杂交。

7.单碱基延伸：双色荧光染料标记的单核苷酸与 gDNA 的互补结合，通过识别“C”和“T”完成微珠探针的末位延伸以及甲基化的判定。甲基化位点通过两种荧光颜色读取区分。

产品名称：Human 27k Beadchip

芯片信息：每张芯片平行检测 12 样本，包含 27,578 个 CpG 位点，12834 个基因来自于 CCDS，1000 多个癌症相关基因、200 个 miRNA 基因启动子区，144 个甲基化热点区。芯片检出率/重复性达 98%以上。

产品名称：Human 450K Beadchip

芯片信息：每张芯片平行检测 12 样本，包含 45 万个 CpG 位点。芯片检出率/重复性达 98%以上。

该芯片设计了 450,000 多个甲基化位点，全面覆盖了 96%的 CpG 岛，并根据需求加入了 CpG 岛以外的 CpG 位点、人类干细胞非 CpG 甲基化位点、正 常组织与肿瘤（多种癌症）组织差异甲基化位点、编码区以外的 CpG 岛、miRNA 启动子区域和已通过 GWAS 的疾病相关区域的位点，同时覆盖了 Human Methylation27 BeadChip 的 90%的位点。每张芯片可平行进行 12 个样本的检测。

产品名称：定制甲基化芯片

针对客户的研究需要提供 96 或者 384 个 CpG 位点进行探针的设计，芯片的定制。

甲基化芯片的定制

Illumina 提 供 ADT（Assay Design Tool）软件用于对客户所提交 CpG 区域进行评价，客户向 ADT 提交 GeneList, RegionList, and SequenceList，illumina 对客户提供的序列进行评价并生成 GGMAScore 文件，客户对 GGMAScore 文件中提到的问题序列进行 修改、删除，再次提交给 ADT，经再次修改后将最终结果提交给 illimina 进行探针的设计和芯片的制备。

Asian J Androl. 2011 Sep;13(5):661-2. doi: 10.1038/aja.2011.90. Epub 2011 Jul 4.

DNA methylome and the complexity of discovering prostate cancer biomarkers.

Koochekpour S.

Genomics. 2011 Aug 2. [Epub ahead of print]

High density DNA methylation array with single CpG site resolution.

Bibikova M, Barnes B, Tsan C, Ho V, Klotzle B, Le JM, Delano D, Zhang L, Schroth GP, Gunderson KL, Fan JB, Shen R.

Abstract

We have developed a new generation of genome-wide DNA methylation BeadChip which allows high-throughput methylation profiling of the human genome. The new high density BeadChip can assay over 480K CpG sites and analyze twelve samples in parallel. The innovative content includes coverage of 99% of RefSeq genes with multiple probes per gene, 96% of CpG islands from the UCSC database, CpG island shores and additional content selected from whole-genome bisulfite sequencing data and input from DNA methylation experts. The well-characterized Infinium® Assay is used for analysis of CpG methylation using bisulfite-converted genomic DNA. We applied this technology to analyze DNA methylation in normal and tumor DNA samples and compared results with whole-genome bisulfite sequencing (WGBS) data obtained for the same samples. Highly comparable DNA methylation profiles were generated by the array and sequencing methods (average R(2) of 0.95). The ability to

determine genome-wide methylation patterns will rapidly advance methylation research.

Br J Cancer. 2011 Aug 9;105(4):575-85. doi: 10.1038/bjc.2011.218. Epub 2011 Jun 28.

Methylator phenotype of malignant germ cell tumours in children identifies strong candidates for chemotherapy resistance.

Jeyapalan JN, Noor DA, Lee SH, Tan CL, Appleby VA, Kilday JP, Palmer RD, Schwalbe EC, Clifford SC, Walker DA, Murray MJ, Coleman N, Nicholson JC, Scotting PJ.

Source

Children's Brain Tumour Research Centre, Centre for Genetics and Genomics, University of Nottingham, Queen's Medical Centre, Nottingham NG7 2UH, UK.

Abstract

Background:Yolk sac tumours (YSTs) and germinomas are the two major pure histological subtypes of germ cell tumours. To date, the role of DNA methylation in the aetiology of this class of tumour has only been analysed in adult testicular forms and with respect to only a few genes.Methods:A bank of paediatric tumours was analysed for global methylation of LINE-1 repeat elements and global methylation of regulatory elements using GoldenGate methylation arrays.Results:Both germinomas and YSTs exhibited significant global hypomethylation of LINE-1 elements. However, in germinomas, methylation of gene regulatory regions differed little from control samples, whereas YSTs exhibited increased methylation at a large proportion of the

loci tested, showing a 'methylator' phenotype, including silencing of genes associated with Caspase-8-dependent apoptosis. Furthermore, we found that the methylator phenotype of YSTs was coincident with higher levels of expression of the DNA methyltransferase, DNA (cytosine-5)-methyltransferase 3B, suggesting a mechanism underlying the phenotype.Conclusion:Epigenetic silencing of a large number of potential tumour suppressor genes in YSTs might explain why they exhibit a more aggressive natural history than germinomas and silencing of genes associated with Caspase-8-dependent cell death might explain the relative resistance of YSTs to conventional therapy.

Cancer Lett. 2011 Dec 1;311(1):29-37. Epub 2011 Jun 24.

Genome-wide combination profiling of DNA copy number and methylation for deciphering biomarkers in non-small cell lung cancer patients.

Son JW, Jeong KJ, Jean WS, Park SY, Jheon S, Cho HM, Park CG, Lee HY, Kang J.

Source

Department of Internal Medicine, Konyang University Hospital, Daejeon 302-718, Republic of Korea.

Abstract

Early detection of lung cancer provides the highest potential for saving lives. To date, no routine screening method enabling early detection is available, which is a key factor in the disease's high mortality rate. Copy number changes and DNA

methylation alterations are good indicators of carcinogenesis and cancer prognosis. In this study, we attempted to combine profiles of DNA copy number and methylation patterns in 20 paired cancerous and noncancerous tissue samples from non-small cell lung cancer (NSCLC) patients, and we detected several clinically important genes with genetic and epigenetic relationships. Using array comparative genomic hybridization (aCGH), statistically significant differences were observed across the histological subtypes for gains at 1p31.1, 3q26.1, and 3q26.31-3q29 as well as for losses at 1p21.1, 2q33.3, 2q37.3, 3p12.3, 4q35.2, and 13q34 in squamous cell carcinoma (SQ) patients, and losses at 12q24.33 were measured in adenocarcinoma (AD) patients ($p<0.05$). In an analysis of DNA methylation at 1505 autosomal CpG loci that are associated with 807 cancer-related genes, we identified six and nine loci with higher and lower DNA methylation levels, respectively, in tumor tissue compared to non-tumor lung tissues from AD patients. In addition, three loci with higher and seven loci with lower DNA methylation levels were identified in tumor tissue from SQ patients compared to non-tumor lung tissue. Subsequently, we searched for regions exhibiting concomitant hypermethylation and genomic loss in both ADs and SQs. One clone representing 7p15.2 (which includes candidate genes such as HOXA9 and HOXA11) and one target ID representing HOXA9_E252_R were detected. Quantitative real-time PCR identified the potential candidate gene HOXA9 as being down-regulated in the majority of NSCLC patients. Moreover, following HOXA9 over-expression, the invasion of representative cell lines, A549 and HCC95, were significantly inhibited. Taken together, our results show that the combined profiling analysis technique is a useful tool for identifying biomarkers in lung cancer and that HOXA9 might be a potential candidate gene for the pathogenesis and diagnosis of NSCLC patients.

Hum Mol Genet. 2011 Jul 15;20(14):2722-33. Epub 2011 Apr 19.

Identification of DNA methylation markers for lineage commitment of in vitro hepatogenesis.

Kim M, Kang TW, Lee HC, Han YM, Kim H, Shin HD, Cheong HS, Lee D, Kim SY, Kim YS.

Source

1Medical Genomics Research Center, Korea Research Institute of Bioscience and Biotechnology, Daejeon, Republic of Korea.

Abstract

Hepatocytes that have differentiated from human embryonic stem cells (hESCs) have great potential for the treatment of liver disease as well as for drug testing. Moreover, in vitro hepatogenesis is a powerful model system for studying the molecular mechanisms underlying liver development. DNA methylation is an important epigenetic mechanism that influences differential gene expression during embryonic development. We profiled gene expression and DNA methylation of three cell states of in vitro hepatogenesis-hESC, definitive endoderm and hepatocyte-using microarray analysis. Among 525 state-specific expressed genes, 67 showed significant negative correlation between gene expression and DNA methylation. State-specific expression and methylation of target genes were validated by quantitative reverse transcription-polymerase chain reaction and pyrosequencing, respectively. To elucidate genome-scale methylation changes beyond the promoter, we also performed high-throughput sequencing of methylated DNA captured by the MBD2 protein. We found dynamic methylation changes in intergenic regions of the human genome during differentiation. This study provides valuable methylation markers for the

lineage commitment of in vitro hepatogenesis and should help elucidate the molecular mechanisms underlying stem cell differentiation and liver development.

Genome Res. 2011 Jul 12. [Epub ahead of print]

A DNA methylation fingerprint of 1628 human samples.

Fernandez AF, Assenov Y, Martin-Subero JI, Balint B, Siebert R, Taniguchi H, Yamamoto H, Hidalgo M, Tan AC, Galm O, Ferrer I, Sanchez-Cespedes M, Villanueva A, Carmona J, Sanchez-Mut JV, Berdasco M, Moreno V, Capella G, Monk D, Ballestar E, Ropero S, Martinez R, Sanchez-Carbayo M, Prosper F, Agirre X, Fraga MF, Graña O, Perez-Jurado L, Mora J, Puig S, Prat J, Badimon L, Puca AA, Meltzer SJ, Lengauer T, Bridgewater J, Bock C, Esteller M.

Source

Cancer Epigenetics and Biology Program (PEBC), Bellvitge Biomedical Research Institute (IDIBELL), L'Hospitalet, 08908 Barcelona, Catalonia, Spain;

Abstract

Most of the studies characterizing DNA methylation patterns have been restricted to particular genomic loci in a limited number of human samples and pathological conditions. Herein, we present a compromise between an extremely comprehensive study of a human sample population with an intermediate level of resolution of CpGs at the genomic level. We obtained a DNA methylation fingerprint of 1628 human samples in which we interrogated 1505 CpG sites. The DNA methylation patterns revealed show this epigenetic mark to be critical in tissue-type definition and stemness, particularly around transcription start sites that are not within a CpG island. For disease, the generated DNA methylation fingerprints show that, during

tumorigenesis, human cancer cells underwent a progressive gain of promoter CpG-island hypermethylation and a loss of CpG methylation in non-CpG-island promoters. Although transformed cells are those in which DNA methylation disruption is more obvious, we observed that other common human diseases, such as neurological and autoimmune disorders, had their own distinct DNA methylation profiles. Most importantly, we provide proof of principle that the DNA methylation fingerprints obtained might be useful for translational purposes by showing that we are able to identify the tumor type origin of cancers of unknown primary origin (CUPs). Thus, the DNA methylation patterns identified across the largest spectrum of samples, tissues, and diseases reported to date constitute a baseline for developing higher-resolution DNA methylation maps and provide important clues concerning the contribution of CpG methylation to tissue identity and its changes in the most prevalent human diseases.