nc886-PKR tumor surveillance pathway in these tumor types, and whether abnormal epigenetic regulation is responsible.

Materials and Methods

Tissue-sample and patient material

A cohort of 100 normal placental tissue, paired cord blood and maternal peripheral blood samples were collected from the Hospital St. Joan De Deu collection. Normal peripheral blood was collected from adult volunteers aged between 19–60 y old. A selection of normal brain samples was obtained from BrainNet Europe/Barcelona Brain Bank and normal kidney samples were obtained from XBTC tissue bank. DNA and RNA extraction and cDNA synthesis were performed as previously described.[25] Ethical approval for adult blood and placenta tissue collection was granted by the Hospital St Joan De Deu Ethics Committee (Study number 35/07) and IDIBELL (PR251/12).

Paired tumor and normal samples sets

The methylation profiles determined by the Illumina Infinium HumanMethylation450 BeadChip arrays for 20 paired normal and bladder tumor samples (urothelial carcinomas), 92 paired breast sample sets (invasive carcinomas), 42 colorectal tumors with adjacent normal tissues (adenocarcinomas) and 69 lung cancers pairs (40 squamous cell carcinomas and 29 adenocarcinomas) were obtained from the Cancer Genome Atlas. Surplus bisulphite DNA from 4 in-house hybridized colorectal cancer sample sets were used for allelic bisulphite PCRs.

Allelic bisulphite sequencing

Prior to methylation analysis all samples were genotyped by PCR and direct sequencing. Sequence traces were interrogated using Sequencher v4.6 (Gene Codes Corporation) to distinguish heterozygous and homozygous samples. Approximately 1 μg DNA was subjected to sodium bisulphite treatment and purified using the EZ GOLD methylation kit (ZYMO). Bisulphite specific primers for each region were used with Hotstar Taq polymerase (Qiagen) for 40 PCR cycles. All PCR products were cloned into pGEM-T Easy vector (Promega) with a minimum of 12 clones were selected for sequencing. All primers for genotyping and bisulphite PCR can found in **Table S3**.

WGBS and Illumina Infinium HumanMethylation450 BeadChip array analysis

We analyzed 6 publically available methylomes, including those derived from CD4+ lymphocytes (GSE31263), brain (GSM913595) liver and placenta (GSE46698). Matching samples were also hybridized to the Illumina Infinium HumanMethylation450 BeadChip arrays (GSE46698). In addition we used 3 leukocyte data sets from GSE30870 and the isolated cell populations published by Reinius and colleagues (GSE35069).[26]

Data filtering and analysis

WGBS: The sequence reads were aligned to either strand of the HG19 reference genome using a custom computational pipeline. The methylation level of each cytosine within CpG dinucleotides was estimated as the number of reads reporting a C, divided by the total number of reads reporting C or T. The location of the *nc886* interval was determined by the sequence from the UCSC sequence browser.

Illumina Infinium HumanMethylation 450 BeadChip array: Before analyzing the data, we excluded possible sources of technical biases that could influence results. We applied signal background subtraction and inter-plate variation was normalized using default control probes in BeadStudio (version 2011.1_ Infinium HD). We discarded probes with a detection P value > 0.01. We also excluded probes that lack signal values in one or more of the DNA samples analyzed. In addition we discarded 16,631 probes as they contained SNPs present in more than 1% of the population (dbSNP 137). We utilized in-house R-package scripts evaluate the methylation of the probes mapping within the chr5: 135414408–135417466 region. The circular heatmaps used to display the DNA methylation profiles of the paired normal and tumor samples were generated using Circos software.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We are grateful to Dr Joan Blanco and Dr Cristina Camprubi for supplying sperm DNA from normal individuals with proven fertility, Professor Hans Keirstead for the phES cell lines and Harry Moore for the SHEF hES cell DNAs. We would like to thank Professor Isidro Ferrer of the Barcelona Brain Bank for supplying human brain specimens. Additional normal tissues were obtained from the Xarxa tumor tissue bank which is sponsored by the Pla Director d'Oncologia de Catalunya (XBTC). This work was supported by the Spanish Ministerio de Educación y Ciencia (BFU2011–27658 to D.M); Fundació La Marató de TV3 (101130 to D.M); Research in the Neonatal Unit in HSJD is partially funded by an unrestricted grant from BBDue Spain. MV is a recipient of a FPU research fellowship and DM is a Ramón y Cajal research fellow (RYC-04548).

Supplemental Materials

Supplemental materials may be found here:
www.landesbioscience.com/journals/epigenetics/article/28323

References

1. Kerkel K, Spadola A, Yuan E, Kosek J, Jiang L, Hod E, Li K, Murty VV, Schupf N, Vilain E, et al. Genomic surveys by methylation-sensitive SNP analysis identify sequence-dependent allele-specific DNA methylation. Nat Genet 2008; 40:904-8; PMID:18568024; http://dx.doi.org/10.1038/ng.174
2. Heyn H, Moran S, Hernando-Herraez I, Sayols S, Gomez A, Sandoval J, Monk D, Hata K, Marques-Bonet T, Wang L, et al. DNA methylation contributes to natural human variation. Genome Res 2013; 23:1363-72; PMID:23908385; http://dx.doi.org/10.1101/gr.154187.112
3. Bourc'his D, Xu GL, Lin CS, Bollman B, Bestor TH. Dnmt3L and the establishment of maternal genomic imprints. Science 2001; 294:2536-9; PMID:11719692; http://dx.doi.org/10.1126/science.1065848
4. Hata K, Okano M, Lei H, Li E. Dnmt3L cooperates with the Dnmt3 family of de novo DNA methyltransferases to establish maternal imprints in mice. Development 2002; 129:1983-93; PMID:11934864
5. Sharif J, Muto M, Takebayashi S, Suetake I, Iwamatsu A, Endo TA, Shinga J, Mizutani-Koseki Y, Toyoda T, Okamura K, et al. The SRA protein Np95 mediates epigenetic inheritance by recruiting Dnmt1 to methylated DNA. Nature 2007; 450:908-12; PMID:17994007; http://dx.doi.org/10.1038/nature06397

6. Smallwood SA, Tomizawa S, Krueger F, Ruf N, Carli N, Segonds-Pichon A, Sato S, Hata K, Andrews SR, Kelsey G. Dynamic CpG island methylation landscape in oocytes and preimplantation embryos. Nat Genet 2011; 43:811-4; PMID:21706000; http://dx.doi.org/10.1038/ng.864
7. Smith ZD, Chan MM, Mikkelsen TS, Gu H, Gnirke A, Regev A, Meissner A. A unique regulatory phase of DNA methylation in the early mammalian embryo. Nature 2012; 484:339-44; PMID:22456710; http://dx.doi.org/10.1038/nature10960
8. Hitchins MP, Rapkins RW, Kwok CT, Srivastava S, Wong JJ, Khachigian LM, Polly P, Goldblatt J, Ward RL. Dominantly inherited constitutional epigenetic silencing of MLH1 in a cancer-affected family is linked to a single nucleotide variant within the 5'UTR. Cancer Cell 2011; 20:200-13; PMID:21840485; http://dx.doi.org/10.1016/j.ccr.2011.07.003
9. Monk D. Deciphering the cancer imprintome. Brief Funct Genomics 2010; 9:329-39; PMID:20551091; http://dx.doi.org/10.1093/bfgp/elq013
10. Jeon SH, Lee K, Lee KS, Kunkeaw N, Johnson BH, Holthauzen LM, Gong B, Leelayuwat C, Lee YS. Characterization of the direct physical interaction of nc886, a cellular non-coding RNA, and PKR. FEBS Lett 2012; 586:3477-84; PMID:22986343; http://dx.doi.org/10.1016/j.febslet.2012.07.076
11. Jeon SH, Johnson BH, Lee YS. A tumor surveillance model: a non-coding RNA senses neoplastic cells and its protein partner signals cell death. Int J Mol Sci 2012; 13:13134-9; PMID:23202942; http://dx.doi.org/10.3390/ijms131013134
12. Treppendahl MB, Qiu X, Søgaard A, Yang X, Nandrup-Bus C, Hother C, Andersen MK, Kjeldsen L, Möllgård L, Hellström-Lindberg E, et al. Allelic methylation levels of the noncoding VTRNA2-1 located on chromosome 5q31.1 predict outcome in AML. Blood 2012; 119:206-16; PMID:22058117; http://dx.doi.org/10.1182/blood-2011-06-362541
13. Blalock WL, Bavelloni A, Piazzi M, Faenza I, Cocco L. A role for PKR in hematologic malignancies. J Cell Physiol 2010; 223:572-91; PMID:20232306
14. Paliwal A, Temkin AM, Kerkel K, Yale A, Yotova I, Drost N, Lax S, Nhan-Chang CL, Powell C, Borczuk A, et al. Comparative anatomy of chromosomal domains with imprinted and non-imprinted allele-specific DNA methylation. PLoS Genet 2013; 9:e1003622; PMID:24009515; http://dx.doi.org/10.1371/journal.pgen.1003622
15. Court F, Tayama C, Romanelli V, Martin-Trujillo A, Iglesias-Platas I, Okamura K, Sugahara N, Simón C, Moore H, Harness JV, et al. Genome-wide parent-of-origin DNA methylation analysis reveals the intricacies of the human imprinting and suggests a germline methylation independent mechanism of establishment. Genome Res 2014; (Forthcoming); http://dx.doi.org/10.1101/gr.164913.113
16. Mai Q, Yu Y, Li T, Wang L, Chen MJ, Huang SZ, Zhou C, Zhou Q. Derivation of human embryonic stem cell lines from parthenogenetic blastocysts. Cell Res 2007; 17:1008-19; PMID:18071366; http://dx.doi.org/10.1038/cr.2007.102
17. Harness JV, Turovets NA, Seiler MJ, Nistor G, Altun G, Agapova LS, Ferguson D, Laurent LC, Loring JF, Keirstead HS. Equivalence of conventionally-derived and parthenote-derived human embryonic stem cells. PLoS One 2011; 6:e14499; PMID:21249129; http://dx.doi.org/10.1371/journal.pone.0014499
18. Monk D, Arnaud P, Apostolidou S, Hills FA, Kelsey G, Stanier P, Feil R, Moore GE. Limited evolutionary conservation of imprinting in the human placenta. Proc Natl Acad Sci U S A 2006; 103:6623-8; PMID:16614068; http://dx.doi.org/10.1073/pnas.0511031103
19. Lim AL, Ng S, Leow SC, Choo R, Ito M, Chan YH, Goh SK, Tng E, Kwek K, Chong YS, et al. Epigenetic state and expression of imprinted genes in umbilical cord correlates with growth parameters in human pregnancy. J Med Genet 2012; 49:689-97; PMID:23042810; http://dx.doi.org/10.1136/jmedgenet-2012-100858
20. Gerdes H, Chen Q, Elahi AH, Sircar A, Goldberg E, Winawer D, Urmacher C, Winawer SJ, Jhanwar SC. Recurrent deletions involving chromosomes 1, 5, 17, and 18 in colorectal carcinoma: possible role in biological and clinical behavior of tumors. Anticancer Res 1995; 15:13-24; PMID:7733622
21. Kambara T, Sharp GB, Nagasaka T, Takeda M, Sasamoto H, Nakagawa H, Isozaki H, MacPhee DG, Jass JR, Tanaka N, et al. Allelic loss of a common microsatellite marker MYCL1: a useful prognostic factor of poor outcomes in colorectal cancer. Clin Cancer Res 2004; 10:1758-63; PMID:15014029; http://dx.doi.org/10.1158/1078-0432.CCR-0779-3
22. Johannsdottir HK, Jonsson G, Johannesdottir G, Agnarsson BA, Eerola H, Arason A, Heikkila P, Egilsson V, Olsson H, Johannsson OT, et al. Chromosome 5 imbalance mapping in breast tumors from BRCA1 and BRCA2 mutation carriers and sporadic breast tumors. Int J Cancer 2006; 119:1052-60; PMID:16570289; http://dx.doi.org/10.1002/ijc.21934
23. Wang ZC, Lin M, Wei LJ, Li C, Miron A, Lodeiro G, Harris L, Ramaswamy S, Tanenbaum DM, Meyerson M, et al. Loss of heterozygosity and its correlation with expression profiles in subclasses of invasive breast cancers. Cancer Res 2004; 64:64-71; PMID:14729609; http://dx.doi.org/10.1158/0008-5472.CAN-03-2570
24. Kunkeaw N, Jeon SH, Lee K, Johnson BH, Tanasanvimon S, Javle M, Pairojkul C, Chamgramol Y, Wongfieng W, Gong B, et al. Cell death/proliferation roles for nc886, a non-coding RNA, in the protein kinase R pathway in cholangiocarcinoma. Oncogene 2013; 32:3722-31; PMID:22926522; http://dx.doi.org/10.1038/onc.2012.382
25. Camprubí C, Iglesias-Platas I, Martin-Trujillo A, Salvador-Alarcon C, Rodriguez MA, Barredo DR, Court F, Monk D. Stability of genomic imprinting and gestational-age dynamic methylation in complicated pregnancies conceived following assisted reproductive technologies. Biol Reprod 2013; 89:50; PMID:23884645; http://dx.doi.org/10.1095/biolreprod.113.108456
26. Reinius LE, Acevedo N, Joerink M, Pershagen G, Dahlén SE, Greco D, Söderhäll C, Scheynius A, Kere J. Differential DNA methylation in purified human blood cells: implications for cell lineage and studies on disease susceptibility. PLoS One 2012; 7:e41361; PMID:22848472; http://dx.doi.org/10.1371/journal.pone.0041361