DNA methylation profiling of low grade astrocytomas using the illumina 450K BeadChip reveals changes in genes involved in brain development and drug resistance

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Summary

- DNA methylation is a crucial regulator of gene expression
- The genome-wide DNA methylation profiles of a large cohort of paediatric low grade astrocytomas have been analysed
- While the majority of genomic sites show similar levels of methylation in pilocytic and diffuse astrocytomas, significant differences are present at specific genes
- Hierarchical clustering according to the differentially methylated regions showed the tumours grouped according to location in the brain
- Genes involved in development and drug resistance show differential methylation, providing new avenues for understanding the biology of low grade astrocytomas

Introduction

Brain tumours are the most common paediatric solid tumours, and are the leading cause of cancer-related death in children under 14 years of age (1,2). Although molecular targeted treatments have been developed for many kinds of cancer, the impact for brain tumours in children has been limited. We are conducting a detailed genetic and epigenetic investigation of a large set of paediatric low-grade astrocytomas to improve our understanding of the biology of these tumours, and identify targets that can be used to develop new forms of treatment.

Astrocytomas are classified into four grades on the basis of biological and histological features (3). Low-grade astrocytomas (grades I & II) predominate in children, while high-grade tumours (grades III & IV) are mainly found in adults. Pilocytic astrocytomas (grade I) are the most common type of astrocytoma in children-aged 0-15, and are well-circumscribed, slow-growing, cystic lesions which are usually successfully treated by surgical excision (4). They can arise throughout the neuraxis, but are usually situated in the cerebellum. In contrast, childhood diffuse astrocytomas (grade II) involve the cerebrum, with infratentorial involvement being most common (5). The key molecular changes identified so far in pilocytic low grade astrocytomas are RAI gene fusions that activate the MAPK signalling pathway in pilocytic astrocytomas (5,6), and abnormalities of the MYB oncogene in certain grade II astrocytomas (7).

To obtain a comprehensive understanding of gene deregulation in paediatric low grade astrocytomas, we have performed a genome-wide study of DNA-methylation in 20 pilocytic astrocytomas and 10 diffuse astrocytomas using the Illumina 450K BeadChip (8). This system measures methylation at >3 consecutive CPG sites across the genome, and is able to detect small changes in CpG island methylation patterns that are not readily apparent in conventional Southern blot analysis. In an unbiased manner, this system allows for the identification of novel candidate genes which may be involved in the aetiology of paediatric low grade astrocytomas.

Method

20 pilocytic astrocytomas and 10 diffuse astrocytomas were obtained as surgical specimens. Age of patients at diagnosis ranged from 3 to 20 years. Access to tumour and linked clinical data was given in accordance with the Health Insurance Portability and Accountability Act (HIPAA) and Institutional Review Board and MREC regulations: St Jude Children’s Research Hospital (USA) XPD07-1071(R), and Tsai Resource Request No 07-037: Newcastle (UK) REC ref No 2002/112; Blizzard Institute (UK) ICM/EPR/GT/77. Control samples were foetal cerebellum, brain and frontal lobe, adult brain and normal telencephalon cell lines (BNChBrain). Normal human astrocyte data was obtained from the UCSC browser. The DNA methylation data was analysed using Illumina Genome Studio software, as well as MultiB, at http://methlab.ccr.ucsf.edu/MultiB.html. Validation was performed using pyrosequencing. Gene expression was analysed using Affymetrix U133 Plus 2.0 arrays, GeneSpring software and RT-PCR.

Results

The overall methylation profiles of pilocytic and diffuse astrocytomas are similar, although there are significant differences at specific genes.

Clustering according to the differentially methylated regions (DMRs) showed the tumours grouped according to location. The paediatric tumours did not show the IDH1-methylator phenotype found in adult-grade II astrocytomas (9).

NRZ21, a gene involved in forebrain development, shows differential DNA methylation within the gene body in supratentorial tumours, which correlates with enhanced expression of the gene.

Conclusion

- Overall methylation profiles of pilocytic and diffuse astrocytomas suggest that these tumours arise from a similar cell type
- Significant differences between pilocytic and diffuse astrocytomas are found in genes involved in CNS development. Developmental processes may thus contribute to the phenotypic disparity between these tumour types
- Alterations of genes involved in drug resistance should be investigated further as a possible means to improve therapeutic efficacy
- Taken together with the recently discovered genetic abnormalities, these epigenetic changes provide a framework for understanding mechanisms of gene deregulation in low grade astrocytomas