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24 Genome-scale DNA methylation analyses of cancer in children

Nicholas C. Wong* and David M. Ashley

24.1 Introduction

Although rare, cancer is a leading cause of disease mortality in children after accidents, homicides, and suicides (Ries et al., 1999). The biology of cancer in children is markedly different to cancer in adults. This is highlighted by the disparate incidence and etiology of common cancers found between children and adults (AIHW and AACR, 2004). In children, leukemia and brain tumors predominate in newly diagnosed cases, while breast, colorectal, melanoma, and lung cancer are common cancers in adults. While there seems to be a genetic basis for adult cancers, where familial cases of breast (reviewed in Narod and Foulkes, 2004) and colorectal (reviewed in Jasperson et al., 2010) cancer have identified mutations of candidate risk genes, the same can not be said for childhood cancer.

The precise causes of childhood cancer still remain unknown. With the scarce number of cases of familial or inherited cancers in children, it would seem that genetics plays a minor role in childhood cancer. Tumours acquire somatic mutation during their progression to cancer and it has long been hypothesized that selected environmental exposures can accelerate mutation rate in tumours that have given rise to their presentation as cancer in children (Knudson, 1976). There is a proposed list of high-risk exposures including radiation, infection, and pesticides associated with childhood cancer (reviewed in Anderson, 2006); however, the small study numbers used to identify these factors have led to small effect sizes and have been difficult to reproduce. Large, multi-center prospective cohort studies have begun to address this issue (Brown et al., 2007).

A number of animal studies linking environmental exposures to changes in epigenetic modifications such as DNA methylation suggest that changes in

* Author to whom correspondence should be addressed.

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phenotype can arise from the environment via changes in the epigenome (reviewed in Sutherland and Costa, 2003). The link between environment and epigenetics is becoming clearer in cancer (reviewed in Ulrich and Grady, 2010). There are an increasing number of studies characterizing DNA methylation changes in cancer with early studies focusing on the promoters of known tumour suppressor and oncogenes. With the advent of second-generation sequencing technology, methods are rapidly being developed for genome-scale characterization of the epigenome (Feinberg, 2010). Genome-scale analysis offers an unbiased approach in cataloging DNA methylation changes associated with disease and could be readily applied here for childhood cancer.

24.2 Methods for DNA methylation analysis

A variety of protocols are available to analyze DNA methylation (summarized in Figure 24.1). Here we will focus on only those used to analyze DNA methylation in childhood cancer, while the remaining protocols are reviewed in Fraga and Esteller (2002). Methylation-specific PCR (MSP) (Herman et al., 1996) is the most widely used loci-specific DNA methylation assay to look at the promoters of known tumour suppressors and oncogenes. A range of methods to look at genome-scale DNA methylation in childhood cancer have been used and these include DNA methylation enrichment, such as methylated DNA immunoprecipitation (MeDIP) and methylated CpG island recovery assay (MIRA), methylation-sensitive restriction enzyme mediated techniques such as HpaII enrichment by ligation-mediated PCR (HELP) and differential methylation hybridization (DMH), and bisulfite-mediated methods such as GoldenGate bead array (reviewed in Laird, 2010).

24.3 Childhood cancer and DNA methylation

Research in childhood cancer has always lagged behind adult cancer. One of the reasons for this is the rarity of cancer in children compared to adults, with an incidence rate of 17.3 cases per 100,000 compared to a rate of 1021.7 per 100,000 (AIHW and ACR, 2004). With such a low incidence in children, sourcing cases and samples for analysis is more challenging for childhood cancers than for adult cancers. As a result, there are only a handful of studies looking at DNA methylation at a genome scale.

Acute leukemia and central nervous system cancers represent the bulk number of tumours in children accounting for 34.1% and 30.2% of diagnosed cases respectively (Kaatsch, 2010). The greatest number of genome-scale DNA methylation analyses and loci-specific DNA methylation studies have been performed for childhood acute lymphoblastic leukemia (Table 24.1). As well as being the most common cancer site in children, bone marrow is easier to access for analysis and manipulation than is the case for brain biopsies.

Acute lymphoblastic leukemia (ALL) describes a disease of the hematopoietic system and involves a block in differentiation of the lymphoid lineage. It is quite heterogeneous with a range of subtypes categorized by common chromosome
Figure 24.1 Overview of methods available for DNA methylation analysis on genomic DNA (based on Fraga and Esteller, 2002). Analysis methods can be divided into three categories: global, genome-scale, and loci-specific DNA methylation methods. Global methods measure DNA methylation of the genome as a whole while genome-scale approaches attempt to analyze most CpG sites across the genome. Loci-specific methods look at DNA methylation at targeted regions/genes within the genome. All genome-scale methods have been employed to investigate DNA methylation in childhood cancer while the most popular loci-specific method used is MSP, HPLC, high-performance liquid chromatography; HPCE, high-performance capillary electrophoresis; LUMA, luminometric methylation assay; ELISA, enzyme-linked immunosorbent assay; DMH, differential methylation hybridization; RE, restriction endonuclease; PCR, polymerase chain reaction; MSP, methylation-specific PCR; MS-SNuPE, methylation-specific single-nucleotide primer extension; COBRA, combined bisulfite and restriction analysis; SSCP, single-stranded conformation polymorphism; HRM, high-resolution melt; RT, in-vitro transcription; MALDI-TOF MS, matrix-assisted laser desorption ionization time of flight mass spectrometry.

aberrations present in the tumour and white cell count on presentation. The difference in distribution of ALL subtypes and relative incidence between adults and children implies that these are two separate diseases with distinct mechanisms of action (Pui et al., 2004). Genomic studies looking for recurrent gene mutations in childhood ALL have accounted for approximately 40% of cases (Mullighan, 2009) suggesting other changes such as DNA methylation could be at play. Differential DNA methylation hybridization (DMH) was one of the first methods used to profile DNA methylation at genome scale (Stumpel et al., 2009).
<table>
<thead>
<tr>
<th>Cancer</th>
<th>Proportion of all cancers</th>
<th>Subtype</th>
<th>DNA methylation protocol</th>
<th>Genome scale</th>
<th>Genes identified</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukemias</td>
<td>34.1%</td>
<td>Acute lymphoblastic leukemia</td>
<td>MedIP</td>
<td>Yes</td>
<td>Over 90 genes</td>
<td>Davidsson et al., 2009</td>
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<td></td>
<td></td>
<td></td>
<td>MIRA</td>
<td>Yes</td>
<td>(reviewed in Chatterton et al., 2010)</td>
<td>Dunwell et al., 2010</td>
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<td></td>
<td></td>
<td></td>
<td>GoldenGate</td>
<td>Yes</td>
<td></td>
<td>Millan et al., 2010</td>
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<td></td>
<td></td>
<td></td>
<td>HELP</td>
<td>Yes</td>
<td></td>
<td>Schafer et al., 2010</td>
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<td></td>
<td></td>
<td></td>
<td>DMH</td>
<td>Yes</td>
<td></td>
<td>Stumpel et al., 2009</td>
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<td></td>
<td></td>
<td></td>
<td>DMH</td>
<td>Yes</td>
<td></td>
<td>Dunwell et al., 2009</td>
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<tr>
<td></td>
<td></td>
<td>Various single genes</td>
<td>No</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Central nervous system cancers</td>
<td>30.2%</td>
<td>Glioma/astrocytoma</td>
<td>MSP</td>
<td>No</td>
<td>MGMT</td>
<td>Buttarelli et al., 2010</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>MedIP</td>
<td>No</td>
<td>RASSF1A</td>
<td>Michalowski et al., 2006</td>
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<td></td>
<td></td>
<td></td>
<td>aPRIMES</td>
<td>Yes</td>
<td>PRDM8, AXIN2, HIC1 and PITCH1</td>
<td>Diele et al., 2010</td>
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<td></td>
<td></td>
<td>Medulloblastoma</td>
<td>DAMD</td>
<td>Yes</td>
<td>ZIC1</td>
<td>Pfister et al., 2007</td>
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<td></td>
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<td>MSP</td>
<td>No</td>
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<td></td>
<td></td>
<td></td>
<td>MSP</td>
<td>No</td>
<td>Caspase-8,</td>
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<td>MSP</td>
<td>No</td>
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<td></td>
<td></td>
<td>MSP</td>
<td>No</td>
<td>DLC1</td>
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<td>Non-Hodgkin's lymphoma</td>
<td>MSP</td>
<td>No</td>
<td>DAPK1</td>
<td>Katzenellenbogen et al., 1999</td>
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<td></td>
<td></td>
<td>MSP</td>
<td>No</td>
<td></td>
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<td>Renal tumors</td>
<td>5%</td>
<td>Wilm's tumour</td>
<td>MSP</td>
<td>No</td>
<td>IGF2/H19</td>
<td>Hubertus et al., 2010</td>
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<td></td>
<td></td>
<td></td>
<td>MeDIP</td>
<td>Yes</td>
<td>PDCD1</td>
<td>Dalloso et al., 2009</td>
</tr>
<tr>
<td>Bone tumors</td>
<td>4.6%</td>
<td>Osteosarcoma</td>
<td>MeDIP/ChIP of cell lines</td>
<td>Yes</td>
<td>WTI, PDCD8B, LHX9, KCND3</td>
<td>Sadjovic et al., 2008</td>
</tr>
<tr>
<td>Hepatic tumors</td>
<td>1.1%</td>
<td>Ewing's sarcoma</td>
<td>MSP</td>
<td>No</td>
<td>RASSF1A</td>
<td>Avigad et al., 2009</td>
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<td></td>
<td></td>
<td></td>
<td>MSP</td>
<td>No</td>
<td>MT1G, APC, CDH1, MT1G, RASSF1A, and SOCS1</td>
<td>Sakamoto et al., 2010</td>
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<td></td>
<td></td>
<td></td>
<td>MSP</td>
<td>No</td>
<td>SOCS1</td>
<td>Sugawara et al., 2007</td>
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<td></td>
<td></td>
<td></td>
<td>MSP</td>
<td>No</td>
<td>RASSF1A, SOCS1 and FGFR1</td>
<td>Nagai et al., 2003</td>
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<td></td>
<td></td>
<td></td>
<td>MSP</td>
<td>No</td>
<td>PAX3</td>
<td>Goldstein et al., 2007</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>MSP</td>
<td>No</td>
<td>MGMT</td>
<td>Kursmacheva et al., 2005</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>MSP</td>
<td>No</td>
<td></td>
<td>Yeager et al., 2003</td>
</tr>
</tbody>
</table>

*MedIP, methylated DNA immunoprecipitation; MIRA, methylated CpG island recovery assay; HELP, HpaII enrichment by ligation-mediated PCR; DMH, differential DNA methylation hybridization; MSP, methylation-specific PCR; DAMD, denaturation analysis of methylation differences; aPRIMES, array-based profiling of reference-independent methylation status; ChIP, chromatin immunoprecipitation.*
and at chromosome 3 (Dunwell et al., 2009). Since then HELP (Schafer et al., 2010), MIRA (Dunwell et al., 2010), and MeDIP (Davidsson et al., 2009) have been used and each has given its own unique list of candidate genes of interest. Numerous other studies in DNA methylation in childhood ALL involve loci-specific approaches such as MSP and are reviewed in more detail in Chatterton et al. (2010). The diversity of genes affected by DNA methylation in childhood ALL is likely due to the range of methods used to profile DNA methylation in childhood leukemia. Furthermore, each study to date has focused on a unique ALL subtype and compared different groups to identify DNA methylation changes of interest. Therefore, no consensus has been reached between studies (Chatterton et al., 2010).

The central nervous system is the next most common site of cancer in children accounting for 30.2% of newly diagnosed cases (Kaatsch, 2010) (Table 24.1). However, there are only a few studies investigating genome-scale DNA methylation (Table 24.1). Denaturation analysis of methylation differences (DAMD) based on the higher melting temperatures of methylated DNA was used to identify common DNA methylation markers associated with medulloblastoma (Diede et al., 2010). The promoters of PRDM8, AXIN2, and HIC1 negative regulators of the Notch, Sonic hedgehog, and Wingless (Wnt) pathways were methylated in medulloblastoma. This was expected because of their known roles in other cancers. The same study identified DNA methylation of a previously uncharacterized upstream promoter of PTCH1 by this method (Diede et al., 2010) and demonstrates the power of genome-scale DNA methylation analysis in identifying novel candidate regions. Another study using genome-scale restriction-enzyme-based microarray analysis in medulloblastoma described differential DNA methylation of the ZIC2 gene promoter (Pfister et al., 2007). Loci-specific studies describe DNA methylation at SFRP (Kongkham et al., 2010) and Caspase-8 (Ebiner et al., 2004) gene promoters in medulloblastoma while P16INK4A, MGMT, TIMP-3, and E-cadherin were unmethylated (Ebiner et al., 2004). The remaining studies in childhood cancer of the central nervous system involve DNA methylation of known tumour suppressors and oncogenes such as MGMT (Buttarelli et al., 2010) and RASSF1A (Michalowski et al., 2006) in childhood glioma. Both studies utilized MSP, and it was found that only a small proportion of cases demonstrated DNA methylation at MGMT (Buttarelli et al., 2010) while up to 60% of cases analyzed were methylated at RASSF1A (Michalowski et al., 2006).

Lymphomas are the next most prevalent form of childhood cancer and to date genome-scale analysis have not been performed on childhood cases while more extensive studies have been performed on adult cases. Gene-specific analyses have described DNA methylation of RASSF1A (Murray et al., 2004) and DLC1 (Ying et al., 2007) in a significant proportion of Hodgkin's lymphoma cases using MSP. Methylation of DAPK1 in non-Hodgkin's lymphoma has also been described with the majority of cases analyzed being methylated at the DAPK1 gene promoter using MSP analysis (Katzenellenbogen et al., 1999).

Renal tumours account for 5% of newly diagnosed cases of childhood cancer with Wilms' tumour (WT) being the major type in children (Kaatsch, 2010).
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Ongoing intensive studies in WT have identified genetic mutations in the WT gene where mutations account for up to 30% of cases (Rutshouser et al., 2008). This tumor is associated with a number of over-growth and under-growth syndromes in which loss of imprinting at the H19 and IGF2 locus is observed (reviewed in Nakamura and Ritchey, 2010). Given the strong linkage between these aberrations, the current focus has been observing DNA methylation changes at imprinted loci with the thinking that loss of imprinting in WT could be a general phenomenon across the genome of WT cases. Further, MeDIP and DNA microarray analysis has been performed on WT cases and a chromosomal region of DNA methylation was identified at 5q31 containing the protocadherin gene cluster (Dallosso et al., 2009). Later work on imprinted loci across the genome confirmed DNA methylation changes at H19 but also revealed by gene expression analyses an associated loss of imprinting at IGF2, NNAT, and MEST (Hubertus et al., 2010).

Bone tumors including osteosarcoma and Ewing’s sarcoma account for 4.6% of childhood cancer cases (Kaatsch, 2010). Tumours are characterized by chromosomal aberrations within the Ewing’s sarcoma gene (EWS) generating fusion proteins with the most common fusion being EWS-FL11 (reviewed in Riggi and Stamenkovic, 2007). EWS-FL11 is thought to behave as an aberrant transcription factor dysregulating downstream gene targets. Limited DNA methylation analysis has been performed in this tumour type with only one description of MeDIP microarray analysis on osteosarcoma model cell lines identifying WTI, PCDHB, LHX9, and KCND3 genes methylated in these cell lines when compared to normal controls (Sadikovic et al., 2008). An MSP analysis of the RASSF1A gene promoter in Ewing’s sarcoma found methylation of RASSF1A was associated with the tumour and a poor prognosis (Avigad et al., 2009).

Hepatoblastoma is the common form of hepatic cancers in children, accounting for 1.1% of cases in children (Kaatsch, 2010). Given their rarity, very few studies in DNA methylation have been performed. All have utilized MSP on candidate gene promoter regions of genes including APC, CDH1, MT1G, RASSF1A, and SOCS1 whose disruption in expression have been implicated in other cancers. In particular, MT1G was found to be associated with poor prognosis in this form of childhood cancer (Sakamoto et al., 2010). Previous studies have also looked at RASSF1A (Sugawara et al., 2007) and SOCS1 (Nagai et al., 2003) in childhood hepatoblastoma with similar findings.

The remaining 14.5% of childhood cancer cases include a wide range of types that individually are very rare in occurrence (Kaatsch, 2010). However, a number of DNA methylation studies have been performed on rhabdomyosarcoma at the gene promoters of FGFR1 (Goldstein et al., 2007), PAX3 (Kurmasheva et al., 2005), and MGMT (Yeager et al., 2003). Bisulfite sequencing was used to describe hypomethylation of the FGFR1 gene promoter and concomitant up-regulation of this gene in tumours (Goldstein et al., 2007). In a similar study, hypomethylation of MGMT promoter was found in the same tumor type using MSP (Yeager et al., 2003) while PAX3 was found to be hypermethylated (Kurmasheva et al., 2005).
24.4 Conclusions and perspectives

The number and scale of studies investigating DNA methylation analysis in childhood cancer seems to decrease with increasing rarity of the cancer type. Cancer is already a rare disease in children and access to high-quality, clinically annotated samples for proper genome-scale analyses will be challenging for the rarest forms of childhood cancer. Given the rarity, one study looked at caspase-8 and caspase-10 gene promoter methylation in a wide range of childhood cancers and found this gene to be highly methylated in various proportions of cases with a particular cancer type (Harada et al., 2002b) while another investigated RASSF1A promoter methylation with similar outcomes (Harada et al., 2002a). With second-generation sequencing technologies maturing, analysis methods exploiting this technology are requiring less sample input for analysis (Feinberg, 2010). There is a great opportunity to study genome-scale DNA methylation in childhood cancer with a properly curated bank of samples. The Cancer Genome Atlas Research Network (2008) is investigating DNA mutations in a range of cancers including glioblastoma multiforme (http://cancergenome.nih.gov/). Moreover, epigenomic studies have recently been completed for this tumor type (Noushmehr et al., 2010). However, childhood cancers have not been included in the Cancer Genome Atlas research program. The International Human Epigenome Consortium (IHEC: www.ihec-epigenomes.org/) and the NIH Epigenome RoadMap (Bernstein et al., 2010) have set out ambitious goals to characterize entire epigenomes in normal and diseased cells; however, there are currently no efforts that are focusing on childhood cancer. The clear differences between adult and childhood cancers preclude direct extrapolation of findings in adult studies to their childhood counterparts. Therefore there is a need to investigate the epigenomes of childhood cancer in addition to current efforts.

REFERENCES


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