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Genetic Factors Influencing the Risk and Clinical Outcome of Neuroblastoma

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1. Introduction

Neuroblastoma is an embryonal malignancy of the sympathetic nervous system arising from neuroblasts. It is the most common solid tumor in children under the age of 5 and accounts for 8-10% of all childhood cancers (Brodeur & Maris, 2006). The disease occurs almost exclusively in infants and children below the age of 4, with median age of diagnosis approximately 17 months (Ries et al., 1999; London et al., 2005). Prognostic factors such as age at diagnosis, clinical stage, Shimada histology, amplification of *MYCN*, DNA ploidy, and molecular defects such as allelic loss of chromosome 1p and 11q in tumor cells are used for risk stratification and treatment assignment. The amplification of *MYCN* oncogene occurs in 20% to 25% of primary neuroblastomas and is consistently associated with poor outcome in neuroblastoma (Brodeur & Seeger, 1986). Although the overall 5-year survival of patients with neuroblastoma have improved considerably over the past decade, survival rates among children with high-risk neuroblastoma remains below 50%, despite marked intensification of chemotherapy (Baade et al., 2010).

A particular hallmark of neuroblastoma is its clinical heterogeneity, where some patients experience spontaneous regression or differentiation of the tumor into benign ganglioneuroma, while others are affected by rapid and fatal tumor progression (Schwab et al., 2003). Although the disease is often diagnosed in the perinatal period, environmental or parental risk factors have not been identified consistently and the molecular basis of neuroblastoma development and progression is still poorly understood (Hamrick et al., 2001; Urayama et al., 2007; Munzer et al., 2008). Recent advances in genome-wide studies have proven to be a useful prognostic tool for identifying genetic alleles or regions that may be used as risk markers for neuroblastoma development. In recent years, a number of genetic and genomic changes have been identified in neuroblastoma tumors that are relevant to clinical progression, allowing tumors to be classified into subsets with distinct clinical behavior. Genome-wide association studies (GWAS) have described genetic factors influencing the risk and clinical outcome of neuroblastoma such as rare mutations in the *ALK* gene for familial neuroblastoma, common single nucleotide polymorphisms (SNPs) at 6p22 in *FLJ22536* and *FLJ44180*, 2q35 in *BARD1*, 11p15.4 in *LMO1*, and copy number variation at 1q21.1 in *NBPF23*. Moreover, several regions with chromosomal alterations have been identified and many of these regions are speculated to harbor tumor suppressor genes.

However, no single genetic change has been found to be common to all neuroblastoma tumors suggesting a complex underlying genetics of neuroblastoma and that aberrant expression or regulation of multiple genes may work together to initiate the malignant transformation of undifferentiated neuroblasts.

More recently, it has become apparent that the biology of neuroblastoma is determined not only by the tumor's genetic profile but also the tumor epigenetic profile. Distinct CpG island methylation patterns have been suggested to characterize different clinical groups of neuroblastoma (Alaminos et al., 2004; Yang et al., 2004; Banelli et al., 2005b; Abe et al., 2007). Indeed, several potential tumor suppressor genes such as *CASP8* and *RASSF1* have been identified to be frequently hypermethylated and silenced in neuroblastoma. Therefore, epigenetic biomarkers may be considered as a potential prognostic marker for predicting risk groups and response to therapy.

This chapter reviews the genetic changes that are associated with the risk and outcome in neuroblastoma with particular focus on recently identified SNPs, copy number variations, genomic changes and epigenetic alterations that have been linked to the tumorigenesis and progression of neuroblastoma. Knowledge of the genetics of neuroblastoma offers opportunities to understand the underlying mechanisms for the heterogeneity of neuroblastoma and will facilitate the discovery of new therapeutic targets.

2. Genetic alterations in neuroblastoma

2.1 *PHOX2B* and *ALK* mutations in familial neuroblastoma

Familial neuroblastoma accounts for approximately 1% of all cases and the disease appears to inherit in an autosomal dominant mode with incomplete penetrance (Maris et al., 2007). Some patients with neuroblastoma have been described with other congenital disorders of neural crest-derived cells, such as central congenital hypoventilation syndrome (CCHS) or Hirschsprung disease (Maris et al., 1997; Amiel et al., 2003). Hence, the co-existence of these disorders and neuroblastoma suggests a common underlying genetic cause. In CCHS, mutations in the paired-like homeobox 2B (*PHOX2B*) gene at chromosome 4p12 is commonly detected, which prompted researchers to examine mutations of *PHOX2B* in neuroblastoma (Weese-Mayer et al., 2005). *PHOX2B* encodes a transcription factor that regulates the development of the autonomic nervous system. Linkage studies of neuroblastoma cases have revealed several germline *PHOX2B* mutations found exclusively in patients with congenital abnormalities of the neural crest (Table 1) (Mosse et al., 2004; Trochet et al., 2004). Although only 6.4% of familial neuroblastoma cases have shown to harbor these mutations, *PHOX2B* was the first gene to be considered as a candidate gene for predisposition to familial neuroblastoma (Raabe et al., 2007). Analysis of *PHOX2B* mutations in sporadic neuroblastoma has also revealed several frameshift mutations (Table 1) (Limpt et al., 2004) and although these mutations occur in less than 3% in sporadic neuroblastomas, they suggest a role for *PHOX2B* in the oncogenesis of neuroblastoma.

For cases that were not associated with other congenital disorders of neural crest development, several groups have independently discovered mutations of the anaplastic lymphoma kinase (*ALK*) gene in familial neuroblastoma as well as sporadic neuroblastoma (Caren et al., 2008; Chen et al., 2008; George et al., 2008; Janoueix-Lerosey et al., 2008; Mosse

et al., 2008). Findings from these studies have shown that the frequency of somatic *ALK* mutations ranged from 4-8% in primary neuroblastoma tumors (Janoueix-Lerosey et al., 2008; Mosse et al., 2008).

Gene	Chromosome location	Gene function	Genetic variations^	References
<i>PHOX2B</i>	4p12	Regulator of autonomic nervous system development	R110L, R141G, G299T, G216fs*88, A241fs*64, G239fs*82	(Limpt et al., 2004; Mosse et al., 2004; Trochet et al., 2004)
<i>ALK</i>	2q23	Encodes for tyrosine kinase	R1275Q, F1275L, F1174L, F1174I, F1174C, F1174V, F1245C, F1245L, F1245V, F1245I, D1091N, A1234T, G1128A, I1171N, I1250T, K1062M, M1166R, R1192P, T1087I, T1151M, Y1278S	(Chen et al., 2008; George et al., 2008; Janoueix-Lerosey et al., 2008; Mosse et al., 2008)
<i>FLJ22536</i> <i>FLJ44180</i>	6p22	Unknown	rs693940, rs4712653, rs9295536	(Maris et al., 2008)
<i>BARD1</i>	2q35	Interacts with <i>BRCA1</i>	rs6435862, rs3768716, rs17487792, rs6712055, rs7587476, rs6715570	(Capasso et al., 2009)
<i>LMO1</i>	11q15.4	Transcriptional regulator	rs110419, rs4758051, rs10840002, rs204938	(Wang et al., 2011)
<i>DUSP12</i>	1q23.3	Encodes for Ser/Thr and Tyr protein phosphatases	rs1027702 ^φ	(Nguyễn et al., 2011)
<i>DDX4</i>	5q11.2	Putative RNA helicases	rs2619046 ^φ	(Nguyễn et al., 2011)
<i>IL31RA</i>	5q11.2	Encodes for type I cytokine receptor family protein	rs10055201 ^φ	(Nguyễn et al., 2011)
<i>HSD17B12</i>	11q11.2		rs11037575 ^φ	(Nguyễn et al., 2011)
<i>NBF23</i>	1q21.1	Unknown	CNV	(Diskin et al., 2009)

^Only missense mutations are listed. ^φMost significant SNP identified.

Table 1. A summary of significant SNPs and CNV at each described predisposition locus identified by GWAS.

The *ALK* gene maps to chromosome 2p23, which also contains *MYCN*, the well-known oncogene in neuroblastoma. The protein product of *ALK* is a tyrosine kinase, an enzyme that regulates the activity of other proteins through phosphorylation. *ALK* plays a critical role in controlling cell proliferation, differentiation and survival in normal cells, especially in the development of the brain and the autonomic nervous system (Wellmann et al., 1997; *The NCBI handbook*, 2002). In many human cancers, *ALK* functions as an oncogene by the activation of *ALK* signaling to form oncogenic fusion proteins through chromosomal

translocation events (Mosse et al., 2009). More than 20 *ALK* mutations have been identified in neuroblastoma patients and cell lines (Table 1), including F1174L, F1174S, F1245C and R1275Q which are located in the conserved regions of the kinase domain and have been shown to activate *ALK* signaling, suggesting their functional importance for the regulation of kinase activity (Chen et al., 2008; Janoueix-Lerosey et al., 2008; Mosse et al., 2008; Martinsson et al., 2011). *ALK* mutations tend to be associated with advanced stage neuroblastoma. In particular, F1174L mutations have been observed to occur at a higher frequency in *MYCN*-amplified tumors, and be associated with poorer outcome, suggesting an interactive role between both aberrations (De Brouwer et al., 2010). Other genetic defects such as amplification and overexpression of the *ALK* gene have been found to correlate with unfavorable features, such as metastatic tumors and poor outcome in neuroblastoma (Caren et al., 2008; Janoueix-Lerosey et al., 2008; Passoni et al., 2009). In addition, the expression levels of *ALK* and *PHOX2B* were directly correlated in neuroblastoma cell lines (Bachetti et al., 2010). Hence, *ALK* has been identified as a novel target gene of *PHOX2B*, indicating that these two genes are jointly involved in the tumorigenesis of neuroblastoma (Bachetti et al., 2010). Since mutations of *ALK* and *PHOX2B* account for the majority of familial neuroblastoma cases, patients with a family history of neuroblastoma are routinely offered genetic counseling and testing for *ALK* and *PHOX2B* mutations (www.ncbi.nlm.nih.gov/sites/GeneTests).

2.2 Genetic variations in sporadic neuroblastoma

The vast majority of neuroblastoma tumors develop sporadically without family history of the disease (Capasso & Diskin, 2010). Genetic variation appears to play a central role in determining neuroblastoma susceptibility with most cases likely to arise from the interaction between multiple genetic variants (Maris et al., 2007). The use of high-density SNP genotyping arrays in GWAS has proven to be a powerful tool in identifying genetic determinants of complex disease. The first report that identified common genetic variants predisposing to neuroblastoma came from a GWAS using blood samples from nearly 2000 neuroblastoma patients and more than 4000 healthy control subjects of European descent (Maris et al., 2008). In this study, over half a million SNPs were genotyped and 3 common SNPs within the *FLJ22536* and *FLJ44180* genes at chromosome 6p22.3 were identified to be associated with the predisposition of sporadic neuroblastoma (Table 1). Investigations also showed that patients that were homozygous for these high-risk alleles were more likely to develop a clinically aggressive form of neuroblastoma, including metastatic neuroblastoma, *MYCN* amplification, and subsequently relapse. Although the function of *FLJ22536* and *FLJ44180* in the tumorigenesis of neuroblastoma is not yet known, these findings suggest that common variants of these two genes may have a distinctive role in the etiology of more aggressive forms of neuroblastoma; a hypothesis examined in subsequent GWAS limited to patients with high-risk neuroblastoma (Capasso et al., 2009). These investigators not only replicated the findings of candidate SNPs at 6p22, a further 6 SNPs within the *BRCA1*-associated RING domain 1 (*BARD1*) gene at 2q35 were found to be associated with aggressive neuroblastoma (Table 1). *BARD1* has been previously implicated to have a role in several types of cancers, including breast cancer. The *BARD1* protein heterodimerizes with *BRCA1* protein and the formation of a stable complex between these proteins is thought to be important for the tumor suppressor function of *BRCA1* (Capasso et al., 2009). However,

further studies are required to characterize the biological consequences of genetic variations in the *BARD1* gene which may lead to the identification of potential therapeutic target for high-risk neuroblastoma.

A further GWAS examining over 2000 patients with neuroblastoma and 6000 control subjects of European ancestry reported that common genetic variants within the *LMO1* gene at 11p15.4 were significantly associated with the risk of neuroblastoma (Table 1) (Wang et al., 2011). *LMO1* encodes a cysteine-rich transcriptional regulator, and its paralogs (*LMO2*, *LMO3* and *LMO4*) have each been implicated in other cancers (Curtis & McCormack, 2010; Wang et al., 2011). Similar to those observed for the 6p22 and *BARD1* loci, the risk alleles of *LMO1* were also found to be associated with high-risk neuroblastoma and decreased survival. In particular, the *LMO1* SNP, rs110419, displayed the strongest association with the aggressive form of the disease. Moreover, presence of the rs110419 variant allele and copy number gains of *LMO1* were associated with increased expression of *LMO1* in neuroblastoma cell lines and primary tumors, suggesting a gain-of-function role of these genetic defects in the tumorigenesis of neuroblastoma (Wang et al., 2011).

More recently, a novel gene-centric approach examined the combined effect of all SNPs within 10 kilobases of 15,885 target genes (Nguyễn et al., 2011). This method correctly identified three genes previously reported to be associated with high-risk neuroblastoma (*FLJ22536*, *BARD1* and *LMO1*). When the analyses were enriched for low-risk neuroblastoma cases, SNPs within four novel genes, dual specificity phosphatase 12 (*DUSP12*), DEAD box polypeptide 4 isoform (*DDX4*), interleukin 31 receptor A precursor (*IL31RA*) and hydroxysteroid (17-beta) dehydrogenase 12 (*HSD17B12*) were identified as being associated with the less aggressive form of neuroblastoma. These susceptibility loci were successfully replicated in two independent cohorts highlighting the importance of robust phenotypic data and the use of alternative methods that focus on individual genes, instead of individual SNPs in GWAS.

Copy number variation (CNV) is another form of genetic variation that has been linked to cancer susceptibility. CNVs are structural variants that comprise of copy number change involving a DNA fragment that is at least one kilobases long (Freeman et al., 2006). Previous investigations identified a deletion CNV at chromosome 1q21.1 that was highly associated with neuroblastoma (Diskin et al., 2009). Sequencing of this region found a previously unknown transcript with high sequence similarity to several neuroblastoma breakpoint family (*NBPF*) genes and this novel transcript was termed *NBPF23* (Diskin et al., 2009). The expression level of *NBPF23* was directly correlated with CNV and *NBPF23* was shown to preferentially express in normal fetal brain and fetal sympathetic tissues, implicating its role in early tumorigenesis of neuroblastoma (Diskin et al., 2009).

2.3 Genomic changes in neuroblastoma

Over the last two decades, many chromosomal and molecular anomalies have been identified in patients with neuroblastoma and the biological and clinical relevance of these genetic changes have been reported. In order to establish reliable genetic markers, several reported molecular defects have been evaluated by the International Neuroblastoma Risk Group (INRG) in a cohort of 8800 neuroblastoma patients to determine their value as a prognostic marker, and some of these markers have been incorporated into risk assessment strategies (Ambros et al., 2009; Cohn et al., 2009).

2.3.1 MYCN amplification and chromosome alterations

The most important of these biologic markers is *MYCN*, an oncogene that is amplified in approximately 20-25% of all neuroblastoma cases and is more common in patients with advanced-stage disease (Brodeur & Seeger, 1986). The process of amplification usually results in 50 to 400 copies of the gene per cell, with correspondingly high levels of *MYCN* protein expression (Seeger et al., 1988). Patients with amplification of *MYCN* tend to have rapid tumor progression and poor prognosis, even in the presence of other favorable factors such as low-stage neuroblastoma. Amplification of *MYCN* is often associated with other chromosomal aberrations such as the deletion of chromosome 1p, which was identified in 25-35% of all neuroblastoma cases (Attiyeh et al., 2005; White et al., 2005). Studies have shown that the addition of an intact human chromosome 1p to a 1p-deleted neuroblastoma cell line can induce cellular differentiation and/or death (Bader et al., 1991), suggesting that the 1p chromosome region harbors tumor suppressor genes (TSGs) or genes that are likely to control neuroblast differentiation. While only a few candidate TSGs have been identified in this region (Okawa et al., 2008), deletion of the 1p region has been associated with unfavorable clinical outcome, independent of age and stage (Caron et al., 1996b) and most 1p-deletions have been found in the 1p36 area of the chromosome; a region showing loss of heterozygosity (LOH) in 20-40% of neuroblastoma tumors (Caren et al., 2007).

Another common chromosomal aberration is the deletion of 11q identified in more than half of all neuroblastoma cases, has found to be highly associated with chromosome 3p LOH (George et al., 2007). As 11q deletions were inversely correlated to *MYCN* amplification, this aberration represents a powerful biomarker of poor outcome in cases without *MYCN* amplification (Attiyeh et al., 2005). Hence, 11q status has recently been included as a criterion in the INRG classification system (Cohn et al., 2009). To a lesser extent, other allelic losses of chromosome segments 3p, 4p, 9p, and 14q have been shown to have varying degrees of prognostic importance (Fong et al., 1992; Caron et al., 1996a; Ejeskar et al., 1998; Vandesompele et al., 1998).

The partial gain of chromosome 17q has been observed in more than 70% of primary neuroblastoma tumors, indicating that a 17q gain is one of the most frequent genetic abnormalities observed in neuroblastoma (Plantaz et al., 1997; George et al., 2007). Unbalanced 17q gain is associated with *MYCN* amplification, loss of 1p, and adverse outcome (Bown et al., 1999). Although this feature may be useful for treatment stratification, the underlying molecular mechanisms conferring the adverse phenotype of neuroblastoma are still unclear.

While recent genome-wide approaches have provided a comprehensive overview of genetic alterations present in neuroblastoma, segmental chromosomal aberrations have also been reported to be associated with clinically aggressive disease and high-risk of relapse (Janoueix-Lerosey et al., 2009; Schleiermacher et al., 2010). In contrast, neuroblastoma patients with whole chromosomal gains or losses have shown better survival and association with favorable clinical disease stage (Lastowska et al., 2001). These findings place a greater emphasis on overall genomic pattern rather than individual conventional markers for inclusion in future treatment stratification system for neuroblastoma.

2.3.2 DNA content

DNA content or ploidy and structural abnormalities, such as chromosomal deletions or gains, have been extensively studied in neuroblastoma. A strong correlation has been found

between increased chromosome number in neuroblastoma cells (diploid versus hyperdiploid) and response to therapy, especially in children less than 1 year of age (Look et al., 1991). While patients with favorable neuroblastoma tend to have a hyperdiploid or near-triploid DNA content (Kaneko et al., 1987), the majority of neuroblastoma cell lines and advanced primary tumors from older patients have either a near-diploid or near-tetraploid DNA content (Maris & Matthay, 1999). Diploid or tetraploid tumors in older patients usually have several structural rearrangements, including amplification, deletion, and unbalanced translocations, while hyperdiploid and triploid tumors in infants generally have whole chromosome gains without structural rearrangements (Kaneko et al., 1987; Maris & Matthay, 1999). These observations are consistent with the findings mentioned earlier that segmental chromosome defects confer a more aggressive phenotype than those with whole chromosome gains or losses.

3. DNA methylation and cancer

Cancer development is an intricate multistep process that involves the malfunction of proto-oncogenes, tumor suppressor genes (TSGs), and other key cellular genes essential for cell differentiation, progression and genome integrity. Malfunction or inactivation of these genes is thought to be predominantly caused by genetic events such as DNA mutations and chromosomal deletions. Until recently, epigenetic alterations were recognized as an alternative mechanism associated with inappropriate gene silencing. Epigenetic changes are heritable alterations in the expression of genes that occur without changing the nucleotide gene sequence of DNA (Das & Singal, 2004). The most well characterized epigenetic event in the mammalian genome is DNA methylation; an essential process that regulates gene transcription and normal cell development. DNA methylation silences gene expression through the addition of methyl groups to cytosine residues within CpG-rich dinucleotides present in the promoter region of genes, where transcription is initiated. Although CpG sites are relatively uncommon in most of the human genome, CpG-rich sequences occurs at a much higher frequency proximal to gene promoter regions and are known as CpG islands (CGIs) and these islands are mostly free of methylation in normal cells (Jones & Baylin, 2002).

In recent years, a growing number of cancer-related genes have been identified to harbor dense methylation in normally unmethylated promoter CGI (Jones & Baylin, 2002). Hypermethylation of the promoter region is often associated with transcriptional silencing of downstream genes such as tumor suppressor genes (Esteller & Herman, 2002). Indeed, many genes implicated in pathways controlling growth, genomic stability and cell survival have been reported to be silenced by promoter hypermethylation. In cancer, gene silencing through methylation occurs at least as frequently as mutations or deletions (Baylin, 2005), while a global decrease in methylated CpG content or hypomethylation is rather uncommon (Kulis & Esteller, 2010). Nevertheless, changes in methylation patterns may lead to chromosomal instability, activation of endogenous parasitic sequences, loss of imprinting, inappropriate expression, aneuploidy, and mutations (Esteller & Herman, 2002). Thus, aberrant methylation is recognized as an important component of tumorigenesis and methylation changes in multiple genes may represent the characteristics of different tumors or tumor subtypes with unique biological and clinical features. Hence, methylation is

considered a promising biomarker for diagnostic and prognostic stratification of cancer patients.

3.1 DNA methylation in neuroblastoma

Although *MYCN* amplification is a strong prognostic marker that identifies a subgroup of patients at high risk of tumor progression and intensive therapy, the majority of metastatic neuroblastomas do not show amplification of this oncogene and these patients can also present with aggressive forms of neuroblastoma (Ambros et al., 2009). Therefore, identification of additional predictive biomarkers is needed for better stratification of patient risk groups and therapeutic regimens.

In the past decade, a growing list of aberrantly methylated genes including those involved in apoptosis, cell-cycle regulation, differentiation and development has been described in neuroblastoma (Table 2). This list is likely to expand as large scale methods for the detection of methylation continue to improve. Despite the current lack of evidence supporting the role of global hypomethylation in neuroblastoma, methylation studies have provided clues for the molecular basis of neuroblastoma and the search for epigenetic signatures that could be associated with defined clinical and biological parameters in neuroblastoma continues. A list of methylation studies and their findings are presented in Table 3. Several studies have found distinct promoter methylation patterns that were able to characterize different clinical groups in neuroblastoma (Abe et al., 2005; Banelli et al., 2005b). The latest findings describing the role of methylation in uncultured or primary neuroblastoma tumors are discussed below.

3.1.1 Tumor suppressor genes

Inactivation of TSGs is a critical step in cancer development. Functional loss of TSGs is usually mediated by oncogenic mutations or chromosomal deletions. In recent years, CGI hypermethylation has been recognized as an alternative mechanism for TSG inactivation and several potential TSGs has been described to be frequently hypermethylated and down-regulated in neuroblastoma.

Allelic losses of chromosome 3p21.3 are frequently detected in many cancers. Several candidate tumor-suppressor genes have been identified in this region, including *RASSF1* (Ras-association domain family 1). This gene encodes for an anaphase inhibitor that prevents cell proliferation by negatively regulating cell-cycle progression through the inhibition of cyclin D1 protein (Nguyễn et al., 2011). Loss or altered expression of *RASSF1* has been associated with the tumorigenesis of other cancers, suggesting the tumor suppressor function of this gene (Burbee et al., 2001). *RASSF1* is consistently methylated in primary neuroblastoma tumors and is frequently inactivated by promoter hypermethylation resulting in loss of expression (Harada et al., 2002; Michalowski et al., 2008). Silencing of *RASSF1* has been postulated to contribute to aberrations of *RAS* signal pathways observed in neuroblastomas (Tanaka et al., 1998). Furthermore, several investigators have reported methylation of *RASSF1* to be associated with unfavorable features. For example, neuroblastoma patients with older age (>1 year) have been shown to have higher levels of *RASSF1* methylation (Harada et al., 2002; Yang et al., 2004), while complete methylation of *RASSF1* has been found to be more prevalent in patients with *MYCN* amplification than

Gene	Gene function	Methylation frequency (%), (no. of samples)	References
<i>APAF1</i>	Proapoptotic gene	28% (23/82)	(Grau et al., 2010)
<i>CASP8</i>	Apoptotic gene, potential TSG	56% (24/42) 60% (21/35) 38% (17/45)	(Hoebeeck et al., 2009) (Lazcoz et al., 2006) (Michalowski et al., 2008)
<i>HOXA9</i>	Development regulator	39% (57/145) [‡]	(Alaminos et al., 2004)
<i>PYCARD</i>	Induces apoptosis	31% (45/145) [‡]	(Alaminos et al., 2004)
<i>RASSF1</i>	Anaphase inhibitor	71% (30/42) 52% (14/27) 70% (39/56) 83% (34/41) 93% (42/45) 84% (26/31) 55% (37/67) 94% (64/68)	(Hoebeeck et al., 2009) (Harada et al., 2002) (Yang et al., 2004) (Lazcoz et al., 2006) (Michalowski et al., 2008) (Banelli et al., 2005b) (Astuti et al., 2001) (Misawa et al., 2009)
<i>SFN</i>	Inhibits cell-cycle progression	100% (31/31)	(Banelli et al., 2005b)
<i>TIMP3</i>	Inhibitor of tissue metallo-proteases, matrix remodeling, tissue invasion	51% (23/45)	(Michalowski et al., 2008)
<i>THBS1</i>	Angiogenesis inhibitor	55% (31/56) 64% (24/38)	(Yang et al., 2004) (Gonzalez-Gomez et al., 2003)
<i>TNRSF10C</i>	Anti-apoptotic decoy receptors	11% (5/45) 21% (6/28)	(Michalowski et al., 2008) (van Noesel et al., 2002)
<i>TNRSF10D</i>	Anti-apoptotic decoy receptors	25% (11/45) 25% (7/28) 42% (13/31)	(Michalowski et al., 2008) (van Noesel et al., 2002) (Banelli et al., 2005b)
<i>ZMYND10</i>	Cell-cycle regulation, potential TSG	15% (6/42) 8% (3/41) 34% (15/45) 41% (20/49)	(Hoebeeck et al., 2009) (Lazcoz et al., 2006) (Michalowski et al., 2008) (Agathangelou et al., 2003)

[‡]Included 27 relapse samples corresponding to the same patients from whom primary tumors were also available.

Table 2. Genes commonly silenced by promoter methylation in primary neuroblastoma tumors.

Gene(s) examined	Detection method	Sample size	Findings	Reference
<i>PTEN</i> , <i>MGMT</i> , <i>PRDM2</i> , <i>hMLH1</i> , <i>CD44</i> , <i>THBS1</i> , <i>GSTP1</i> , <i>CFTR</i> , <i>TNFRSF10A</i> , <i>ZMYND10</i> , <i>RASSF5</i> , <i>RARβ</i> , <i>CASP8</i> , <i>PYCARD</i> , <i>APAF1</i> , <i>RB1</i> , <i>EMP3</i> , <i>CCND2</i> , <i>RASSF1</i> , <i>SYK</i>	MSP	82	Hypermethylation of <i>CASP8</i> , <i>PYCARD</i> and <i>THBS1</i> were associated with <i>MYCN</i> amplification and poor EFS and OS. Combined analysis of hypermethylation of apoptotic genes (<i>CASP8</i> , <i>PYCARD</i> and <i>APAF1</i>) was suggested as a good prognostic indicator of NB progression.	(Grau et al., 2010)
<i>SFN</i>	Pyro-sequencing & MSP	122	A methylation threshold of 85% for the <i>SFN</i> gene distinguished NB patients with progressive disease from those with favorable outcome.	(Banelli et al., 2010)
<i>ROBO1</i> , <i>PRMD2</i> , <i>TP73</i> , <i>DCC</i> , <i>CDH1</i> , <i>ZMYND10</i> , <i>PTEN</i> , <i>CASP8</i> , <i>RASSF1</i> , <i>CD44</i>	MSP	42	Hypermethylation of <i>CASP8</i> and <i>CDH1</i> was associated with poor EFS. Meta-analysis of 115 NB tumors demonstrated that <i>CASP8</i> methylation and <i>MYCN</i> amplification are correlated.	(Hoebeeck et al., 2009)
<i>RASSF1</i>	MSP	68	Hypermethylation of <i>RASSF1</i> was found in 94% of NB tumors and 25% in matched serum samples. Serum methylation of <i>RASSF1</i> was associated with age at diagnosis (≥ 1 year), stage 4 NB and <i>MYCN</i> amplification.	(Misawa et al., 2009)
<i>TIMP3</i> , <i>CASP8</i> , <i>ZMYND10</i> , <i>TNFRSF10C</i> , <i>TNFRSF10D</i> , <i>CDKN2B</i> , <i>RARβ</i> , <i>DAPK1</i> , <i>FHIT</i> , <i>NF2</i> , <i>CDKN2A</i> , <i>CDKN2A</i> , <i>APC</i> , <i>RB1</i> , <i>SMARCB1</i> , <i>NF2</i> , <i>CFLAR</i> , <i>CDH1</i> , <i>MGMT</i>	MSP	45 & 17 relapse	Methylation of <i>RASSF1</i> , <i>TIMP3</i> , <i>CASP8</i> , <i>ZMYND10</i> was detected at diagnosis as well as relapse and were associated with unfavorable stage.	(Michalowski et al., 2008)
<i>HIC1</i> , <i>PYCARD</i> , <i>TNFRSF10D</i> , <i>ZMYND10</i> , <i>TNFRSF10A</i> , <i>CDH1</i> , <i>SCGB3A1</i> , <i>RARRES1</i> , <i>IRF7</i> , <i>CDH13</i> , <i>EDNRB</i> , <i>MGMT</i> , <i>BRCA1</i> , <i>RB1</i> , <i>P27</i> , <i>DKK3</i> , <i>VHL</i> , <i>SLC16A1</i> , <i>PTEN</i>	MSP	70	<i>TNFRSF10D</i> , <i>CASP8</i> and <i>SCGB3A1</i> were associated with high-risk NB and poor outcome.	(Yang et al., 2007)
<i>RASSF1</i> , <i>RASSF5</i> , <i>ZMYND10</i> , <i>CASP8</i>	MSP	41	A correlation was found between the methylation levels of <i>RASSF1</i> and <i>CASP8</i> . No association was detected between methylation and known prognostic factors.	(Lazcoz et al., 2006)
<i>PCDHB</i> gene family, <i>PCDHA</i> gene family, <i>MST1</i> , <i>MST1P9</i> , <i>DKFZp451I127</i> , <i>FBLN7</i> , <i>ZBTB22</i> , <i>CYP26C1</i>	MS-RDA & MSP	145	Multiple CGIs were simultaneously methylated in patients with poor prognosis, conforming to the concept of CpG island methylator phenotype (CIMP). Almost all cases with <i>MYCN</i> amplification exhibited CIMP.	(Abe et al., 2005)

Gene(s) examined	Detection method	Sample size	Findings	Reference
<i>SFN</i> , <i>RASSF1</i> , <i>CDKN2A</i> , <i>CDH1</i> , <i>CASP8</i> , <i>TNFRSF10D</i> , <i>RARβ</i> , <i>TNFRSF10A</i> , <i>MGMT</i> , <i>TAp73</i> , $\Delta Np73$	MSP, COBRA & direct sequencing	31	Methylation of <i>SFN</i> , <i>RASSF1</i> and intragenic segment of <i>CASP8</i> was different between <i>MYCN</i> amplified and non-amplified NB tumors. Hypermethylation of <i>TNRSF10D</i> was associated with reduced overall survival.	(Banelli et al., 2005b)
<i>TNFRSF10A</i> , <i>PYCARD</i> , <i>RARβ</i> , <i>SYK</i> , <i>PRDM2</i> , <i>FOLH1</i> , <i>CDKN2A</i> , <i>CCND2</i> , <i>LMX1A</i> , <i>HOXA9</i>	MSP	118 & 27 relapse	Hypermethylation of <i>HOXA9</i> was associated with poor survival in patients <1 year of age and with no amplification of <i>MYCN</i> .	(Alaminos et al., 2004)
<i>SPARC</i> , <i>TIMP3</i> , <i>THBS1</i> , <i>DAPK1</i> , <i>TP73</i> , <i>FAS</i> , <i>CDKN2A</i> , <i>CDKN1A</i> , <i>RASSF1</i> , <i>RARβ2</i> , <i>CASP8</i>	MSP	56	Hypermethylation of <i>RASSF1</i> was associated with age >1 year, high-risk NB and poor survival. No association between <i>THBS1</i> methylation and prognostic factors or survival was observed.	(Yang et al., 2004)
<i>ZMYND10</i> , <i>RASSF1</i>	MSP & COBRA	49	Methylation of <i>ZMYND10</i> was detected in 41% of primary NB tumors. No correlation was found between methylation of <i>RASSF1</i> and <i>ZMYND10</i> .	(Agathangelou et al., 2003)
<i>MGMT</i> , <i>DAPK1</i> , <i>CDKN2A</i> , <i>THBS1</i> , <i>TIMP3</i> , <i>TP73</i> , <i>CDKN2A</i> , <i>RB1</i> , <i>CASP8</i> , <i>TP53</i> , <i>GSTP1</i>	MSP	38	A high frequency of methylation (64%) was detected in <i>THBS1</i> , while all other genes have intermediate or low methylation frequency (0-30%).	(Gonzalez-Gomez et al., 2003)
<i>TNFRSF10A</i> , <i>TNFRSF10B</i> , <i>TNFRSF10C</i> , <i>TNFRSF10D</i>	MSP	28	Hypermethylation was observed for <i>TNFRSF10C</i> and <i>TNFRSF10D</i> , while <i>TNFRSF10A</i> and <i>TNFRSF10B</i> were frequently expressed in NB tumors with no methylation was observed.	(van Noesel et al., 2002)
<i>CDKN2A</i> , <i>MGMT</i> , <i>GSTP1</i> , <i>RASSF1</i> , <i>APC</i> , <i>DAPK1</i> , <i>RARβ2</i> , <i>CDH1</i> , <i>CDH13</i>	MSP	27	Methylation of <i>RASSF1</i> had the highest frequency (52%) when compared to other genes (<6%). Hypermethylation of <i>RASSF1</i> was associated with age >1 year.	(Harada et al., 2002)
<i>RASSF1</i> , <i>CASP8</i>	MSP & COBRA	67	<i>RASSF1</i> was methylated in the majority of primary NB tumors and <i>RASSF1</i> promoter methylation is was associated with transcriptional silencing of <i>RASSF1</i> in NB cell lines. Methylation of <i>RASSF1</i> and <i>CASP8</i> was found to be correlated.	(Astuti et al., 2001)

Abbreviation: MSP = methylation specific PCR; COBRA = combined bisulfite restriction analysis; MS-RDA = methylation-sensitive representational difference analysis; EFS = event-free survival; OS = overall survival; CGI = CpG island; NB=neuroblastoma.

Table 3. A summary of findings from methylation studies in primary neuroblastoma tumors.

those without (Banelli et al., 2005b). However, associations between *RASSF1* methylation and clinical outcome of neuroblastoma have been variable. One study found significant association between *RASSF1* methylation and high-risk neuroblastoma as well as poor survival (Yang et al., 2004), while other studies were unable to detect any associations (Harada et al., 2002; Wong et al., 2004; Lazcoz et al., 2006). Nevertheless, when the combined methylation levels of both *RASSF1* and *TNFRSF10D* are considered, their clinical association with reduced overall survival and progressive tumors becomes more apparent (Banelli et al., 2005b). Similarly, methylation patterns in *RASSF1* and *CASP8* have been reported to be correlated, although the clinical significance of this association is yet to be established (Astuti et al., 2001; Lazcoz et al., 2006). More recently, a study examining the level of promoter hypermethylation of *RASSF1* in serum DNA samples of patients with neuroblastoma found increased levels of *RASSF1* hypermethylation associated with older age, stage 4 disease, and *MYCN* amplification (Misawa et al., 2009). These promising findings indicate that screening for methylation status of *RASSF1* and other genes in patient serum at diagnosis may be further developed for use as a non-surgical prognostic predictor of neuroblastoma outcome.

ZMYND10 (zinc finger, MYND-type containing 10, also known as *BLU*) is another candidate tumor suppressor gene residing in the 3q21 region, and is thought to regulate entry into the cell cycle. Overexpression of *ZMYND10* has been shown to inhibit cell growth in neuroblastoma, while methylation of the *ZMYND10* promoter has been correlated with reduce *ZMYND10* gene expression in neuroblastoma cell lines (Agathangelou et al., 2003) and hypermethylation of *ZMYND10* has been reported in a broad spectrum of tumors including neuroblastoma (Agathangelou et al., 2003; Qiu et al., 2004). Methylation of *ZMYND10* has been shown to be associated with clinical stage, with stages 1, 2, and 4S showing significantly less methylation than stages 3 and 4 (Michalowski et al., 2008). An association between *ZMYND10* methylation and *MYCN* amplification has also been reported but the underlying mechanism for this is yet to be determined (Hoebeeck et al., 2009). Although *ZMYND10* is located immediately upstream of *RASSF1*, no correlation has been found between the methylation levels of these two genes in neuroblastoma, suggesting that methylation or inactivation of *ZMYND10* is an independent event and does not result from a common deleted region (Agathangelou et al., 2003).

3.1.2 Apoptosis-related genes

Neuroblastoma has the highest rate of spontaneous regression among other malignant tumors (Hero et al., 2008). The molecular basis of spontaneous regression is often explained by the ability of neuroblastoma cells to differentiate into ganglion cells or to delay activation of apoptosis (Oue et al., 1996). Apoptosis is a process of programmed cell death dependent on the coordinated control of multiple highly conserved genes that leads to cell disruption. Alterations in the apoptosis pathway have been implicated in several aspects of tumor cell growth. Indeed, the level of expression in molecules involved in apoptosis has been shown to be a prognostic factor in patients with neuroblastoma (Islam et al., 2000; Casciano et al., 2004; Takita et al., 2004).

Methylation of the pro-apoptotic gene *PYCARD* (PYD and CARD domain-containing protein, also known as *TMS1*), has been reported in patients with advanced stage neuroblastoma, while no evidence of hypermethylation of *PYCARD* was found in patients

with spontaneous regression (Alaminos et al., 2004; Grau et al., 2010). *PYCARD* induces apoptosis, and inhibits tumor cell survival. Hence its silencing via methylation could confer a growth advantage for tumor cells allowing escape from the apoptotic process (Banelli et al., 2005a). The absence of *PYCARD* expression driven by methylation has been demonstrated in other cancers (Martinez et al., 2007; Zhang et al., 2007).

Similar to the *PYCARD* gene, hypermethylation of the *APAF1* (apoptotic peptidase activating factor 1) gene has been reported to be associated with poorer prognosis in neuroblastoma patients (Grau et al., 2010). This gene has been described as a pro-apoptotic gene and a putative TSG in *MYCN* amplified neuroblastoma (Teitz et al., 2002). *APAF1* is a cytoplasmic protein that initiates apoptosis through activation of caspase-9 (Hausmann et al., 2000). Hence, silenced expression of *APAF1* through hypermethylation could dampen the initiation of the caspase cascade, thereby reducing the apoptotic activity of the gene.

The *CASP8* (caspase-8) gene is located at chromosome band 2q33, a region associated with LOH in neuroblastomas and several other tumor types (van Noesel & Versteeg, 2004). This gene encodes for cysteine protease, a key enzyme at the top of the apoptotic cascade and is activated in programmed cell death. Down-regulation of *CASP8* is one of the most well-known apoptotic defects in neuroblastoma. Indeed, it has been shown that the loss of *CASP8* expression was highly correlated with the amplification of *MYCN* (Teitz et al., 2000). Hypermethylation of *CASP8* has frequently been reported in neuroblastoma and the aberrant methylation of this gene is often associated with *MYCN* amplification (Teitz et al., 2000; Casciano et al., 2004; Hoebeeck et al., 2009). However, structural analysis of *CASP8* has revealed that the region showing differential methylation patterns between *MYCN*-amplified and non-amplified tumors was an intragenic sequence between exons 2 and 3 in the *CASP8* gene which lacked promoter activity (Banelli et al., 2002). Although subsequent studies have identified a *CASP8* promoter, the effect of DNA methylation in the promoter region of *CASP8* has not been shown to have a direct impact on gene expression (Banelli et al., 2002; Banelli et al., 2005a). Nonetheless, neuroblastoma cell lines treated with demethylation agent 5-aza-2' deoxycytidine (5-AZA) activates *CASP8* expression, suggesting that demethylation of a trans-acting factor or gene controls the activity of *CASP8* (van Noesel, 2004).

TRAIL (tumor necrosis factor-related apoptosis-inducing ligand) is a member of the tumor necrosis factor (TNF) family of ligands capable of initiating apoptosis in a variety of cancer cells but not in most normal cells (van Noesel et al., 2002). Apoptotic signaling of TRAIL is induced by interacting with its death receptors (DRs) encoded by *TNFRSF10A* and *TNFRSF10B* genes. However, two anti-apoptotic decoy receptors (DcRs) encoded by *TNFRSF10C* and *TNFRSF10D* genes, compete with death receptors for binding to TRAIL and prevent normal cells from TRAIL-mediated apoptosis (van Noesel et al., 2002). Hence, the balanced expression of all four receptors is required to prevent TRAIL-induced apoptosis in normal cells.

Methylation of the death receptors, *TNFRSF10A* and *B*, has not been detected in primary neuroblastoma tissue and these receptors are frequently expressed (van Noesel et al., 2002). However, DcR proteins encoded by the *TNFRSF10C* and *D* genes are silenced by promoter methylation in a variety of tumors including neuroblastoma (van Noesel et al., 2002; Banelli et al., 2005b). Methylation of *TNFRSF10D* has been shown to be associated with reduced overall survival in neuroblastoma patients independent of *MYCN* amplification, suggesting that aberrant methylation of *TNFRSF10D* may be a potential prognostic biomarker for

unfavorable outcome (Banelli et al., 2005b; Yagyu et al., 2008). In addition, the strong correlation between methylation of *TNFRSF10D* in sera and in neuroblastoma tumors, further supports the possibility of using serum measures of gene methylation as prognostic markers for clinical outcome in neuroblastoma (Yagyu et al., 2008). However, the biological significance of *TNFRSF10D* in carcinogenesis remains unclear.

3.1.3 Cell cycle, signal transduction and other genes

THBS1 (thrombospondin-1, also known as *TSP-1*), is a well-known inhibitor of angiogenesis and altered expression of *THBS1* is thought to contribute to neo-vascularization and metastasis in human cancer (Roberts, 1996). Studies have shown that *THBS1* promoter is frequently methylated and silenced in neuroblastoma (Gonzalez-Gomez et al., 2003; Yang et al., 2003). However, there has been an absence of any association detected between methylation of *THBS1* and clinical features such as *MYCN* amplification, deletion of 1p in neuroblastoma, and tumor type (Yang et al., 2003).

The *SFN* (stratifin, also known as 14.3.3 δ) gene is directly regulated by p53 and is thought to function as a G2/M phase cell-cycle regulator by inhibiting cell-cycle progression, causing cells to leave the stem-cell compartment and undergo differentiation (Hermeking, 2003). Inactivation of *SFN* has been shown to be involved in tumor development in a variety of malignant tumors (Hermeking, 2003) with demethylation of the *SFN* promoter significantly increasing the expression of this gene in neuroblastoma (Banelli et al., 2010). *SFN* has been found to be fully methylated in *MYCN*-amplified neuroblastoma and partially methylated in non-amplified tumors (Banelli et al., 2005b). More recently, quantitative pyrosequencing analysis has identified that a methylation threshold level of 85% for the *SFN* gene distinguishes neuroblastoma patients presenting with progressive disease from those with a more favorable outcome, independent of other prognostic markers (Banelli et al., 2010).

The *HOXA9* (homeobox A9) gene encodes a sequence-specific transcription factor which is part of a developmental regulatory system that provides cells with specific positional identities on the anterior-posterior axis of an organism (*The NCBI handbook*, 2002). Dysregulated expression of *HOXA9* has been described in several malignancies including non-small-cell lung cancer (Calvo et al., 2000) and breast cancer (Gilbert et al., 2010). Neuroblastoma cell lines treated with demethylating agents have been reported to display increased levels of *HOXA9* gene expression (Margetts et al., 2008). Comprehensive methylation profiling of a large series of neuroblastoma tumors has shown that promoter hypermethylation of *HOXA9* is associated with poorer survival of patients aged ≥ 1 year and patients without *MYCN*-amplification (Alaminos et al., 2004). Currently, no clinical or pathologic prognostic markers have been identified for these two groups of patients. Hence, *HOXA9* methylation may be a useful biomarker that can predict the clinical outcome of these subgroups.

3.1.4 *MYCN* and methylation

As mentioned earlier, numerous reports have demonstrated that hypermethylation of certain tumor-related genes such as *CASP8*, *RASSF1*, and *ZMYND10* is most evident in *MYCN*-amplified neuroblastomas. Although these observations may have occurred by chance, there may be additional mechanisms driving the methylation of certain genes in tumors with *MYCN*-amplification. *MYCN* encodes for a transcription factor that binds to recognition sites such as E-box promoter sequence of target genes to activate the

transcriptional activity of the associated genes. *c-MYC*, a functional homolog of *MYCN*, does not appear to bind to recognition sequences that include a methylated CpG, resulting in transcriptional repression and *MYCN* could interact in a similar manner (Prendergast & Ziff, 1991). Another possible explanation is that epigenetic alterations may have a specific role in more aggressive subtypes of neuroblastoma. This hypothesis is supported by observations from a genome-wide screen of neuroblastoma tumor samples where the methylation of multiple CGIs of particular genes were dependent upon each other and this phenotype was significantly associated with poor survival and *MYCN* amplification (Abe et al., 2005). These findings indicate that some genes may become methylated in a coordinated manner, suggesting a “CpG islands methylator phenotype” (CIMP) which was originally recognized in colorectal cancer (Abe et al., 2005). Recent evidence supporting the presence of CIMP in neuroblastoma comes from a genome-wide DNA methylation analysis of neuroblastoma tumors identifying large-scale blocks of contiguously hypermethylated CGIs, with a highly biased distribution towards the telomeric or terminal regions of the chromosome (Buckley et al., 2011). The aberrant methylation of multiple genes giving rise to distinctive neuroblastoma tumors or tumor subtypes may explain the biologically and clinically variable features observed in neuroblastoma. Furthermore, clustering of methylation data from neuroblastoma cell lines distinguished those with *MYCN* amplification from others (Alaminos et al., 2004). Therefore, it is possible that both *MYCN* amplification and CIMP contribute to a more aggressive type of neuroblastoma and the detection of methylation of certain genes in the aggressive type of neuroblastoma coincided with *MYCN* amplification. Taken together, the molecular mechanism for *MYCN* and methylation is still unclear and warrants further studies.

3.2 Considerations for future methylation analysis

While there have been many reports demonstrating gene inactivation driven by DNA methylation in neuroblastoma, the frequency of methylation varies considerably between different studies. The observed variation is likely to reflect the genetic heterogeneity of neuroblastoma, where primary tumors are comprised of multiple cell types such as the S-type (substrate adherent), N-type (neuroblastic), and I-type (stem) cells; with each type of cell having a distinct methylation and gene expression profile (Alaminos et al., 2004). Hence, inherent variability may not reflect the real differences in hypermethylation profiles of primary tumors but distinct cell types. Moreover, neuroblastoma is a cancer of the developing neural crest in which several of the cell types are pluripotent and have the capacity to differentiate into other neuroblastoma cell types. Thus, differences that are seen in hypermethylation profiles in a particular neuroblastoma cell may reflect changes in methylation of a normal differentiating cell rather than development of a cancer phenotype (Ross & Spengler, 2004). Future studies may benefit from incorporating immunocytochemical studies to identify the proportion of each cell type and evaluate the level of methylation to the particular cell type accordingly. The use of different techniques for detecting DNA methylation presents another source of variation. Hence, standardized methods and scoring systems should be established for more comparable results between laboratories.

Over the past decade, an increasing number of genes are discovered to be epigenetically silenced in tumors. Methylation analysis is rapidly progressing from the study of a single or few genes into that of the high-throughput determination of the methylation status of

thousands of CGIs by microarray analysis. Similar limitations of GWAS also apply to the genome-wide search for epigenetic markers. The large number of comparisons performed increases the error. Therefore, adjustment for multiple-testing should be considered such as the use of false discovery rate for the identification of as many true associations as possible while minimizing the overall proportion of false-positive tests (Foley et al., 2009).

Whether a candidate gene or genomic approach is used, studies should aim to identify genes with promoter CGI hypermethylation that results in subsequent gene silencing. Although demethylating agents are commonly used in studies to identify genes that are reactivated, using demethylating agents alone is not a definite proof that the gene has methylation-associated silencing since gene expression can be indirectly induced through other transcriptional factors that are epigenetically controlled. A more effective plan of investigation might be to first identify genes with CGI hypermethylation, then test for the functionality of methylation using demethylating agents. When treating cell lines with demethylating agents, it is important to include a control cell line with low or no methylation and assess the level of candidate gene expression pre- and post- treatment. Change in expression in the control cell line indicates that other transcriptional activators were methylated and that expression is not due to methylation-induced silencing of the gene. In addition, it is also possible that candidate TSGs that are unmethylated but upregulated by demethylating agents may be indirect markers for downstream epigenetically inactivated TSGs.

Although methylated promoter CGIs generally disable the transcription of the correlated gene, other concomitant epigenetic events such as changes in histone proteins may affect DNA organization and gene expression. Changes in chromatin structure also influence gene expression as genes are inactivated when the chromatin is condensed and expressed when the chromatin is in an open configuration (Rodenhiser & Mann, 2006). These dynamic chromatin states are controlled by histone modifications, involving the histone deacetylase (HDACs) family of enzymes in this reversible epigenetic process. Active promoter regions normally have unmethylated DNA and high levels of acetylated histones, while inactive regions of chromatin contain methylated DNA and deacetylated histones. Therefore, a full evaluation of promoter DNA hypermethylation, histone modification and quantitative gene expression will help to decipher the entire epigenome. The International Human Epigenome Project (IHEP), is an international collaboration that aims to identify, catalogue and interpret genome-wide DNA methylation patterns of all human genes in all major tissues. This project will provide high-resolution reference epigenome maps to the research community (The American Association for Cancer Research Human Epigenome Task Force; The European Union Network of Excellence Scientific Advisory Board, 2008). These maps will integrate the various epigenetic layers of detailed DNA methylation, histone modification, nucleosome occupancy and expression patterns of coding and non-coding RNA in different normal and disease cell types which will be a rich source of information for the study of tumorigenesis and for the identification of cancer-specific methylation biomarkers.

4. Future directions: Clinical implications of DNA methylation in neuroblastoma

In the next few years, an increasing number of novel biomarkers for neuroblastoma will continue to be identified through epigenomic profiling. This approach will not only help further understand the molecular mechanisms governing neuroblastoma, the clinical

relevance of these novel biomarkers will also serve to stratify tumor types, identify prognostic groups, predict therapeutic response and assess the risk of relapse. As DNA methylation patterns are relatively easy to detect and specific to tumor types, specific methylation patterns may be useful in the clinical setting. In addition, studies have accurately detected aberrant methylation of particular genes in biological fluids such as serum, sputum, or urine which will allow early diagnosis of cancer without the need for invasive surgery. However, the sensitivity and specificity of DNA methylation markers in cancer diagnosis depends on tumor type, the gene studied, the type of body fluid used, and the technique involved. Therefore, DNA methylation detection methods need to become more standardized to facilitate sensitive, accurate and reproducible results in the clinical setting. To date, studies examining the relationship between DNA methylation and individual treatment response in neuroblastoma are limited. Moreover, the DNA methylation profiling may also be useful in the continued assessment of patients throughout treatment.

Unlike genetic alterations, DNA methylation can be reversed to restore the function of key control pathways in malignant and premalignant cells by treatment with demethylating agents. DNA methylation inhibitors such as azacitidine and decitabine can induce functional re-expression of aberrantly silenced genes in cancer, causing growth arrest and apoptosis in tumor cells (Jones & Baylin, 2002). More recently, several inhibitors of chromatin-modifying enzymes, including histone deacetylase (HDAC) inhibitors and DNA methyltransferase (DNMT) inhibitors have now been approved by US Food and Drug Association (FDA) and are being used in clinical practice with good prognosis for tumor regression. For example, DNMT inhibitors such as 5-azacytidine (Vidaza®) and decitabine (Dacogen™) have been approved for the treatment of myelodysplastic syndrome and leukemia (Mack, 2006). However, the treatment of solid tumors with DNMT inhibitors showed response rates of less than 10% and is considerably less successful than the treatment of leukemias (Goffin & Eisenhauer, 2002). Recently, decitabine was used in a phase I clinical trial as an anticancer drug for children with solid tumor and neuroblastoma (George et al., 2010). Although patients had tolerable toxicity to low-dose decitabine in combination with doxorubicin/cyclophosphamide, doses of decitabine capable of producing clinically relevant biologic effects were not well tolerated with this combination. Therefore, further studies are required to examine the efficacy of HDAC and DNMT inhibitors in combination with current treatment protocols to identify best treatment options for neuroblastoma.

5. Conclusion

It is clear that both genetic and epigenetic changes play a crucial role in the tumorigenesis of neuroblastoma. The genetic heterogeneity of neuroblastoma suggests that the initiation and progression of this disease requires multiple interacting genetic factors including genetic variants in susceptibility loci, copy number variations, amplification of oncogenes, deletion of tumor suppressor genes, and other genetic mechanisms such as DNA methylation. These genetic events may act alternatively or synergistically in the multistep process of carcinogenesis. With recent technological advances in whole-genome microarrays, both genetic and epigenetic screens should be undertaken to enumerate the full spectrum of alterations in the human cancer genome to facilitate the identification of novel biomarkers for the most efficient grouping of neuroblastoma. More importantly, it will direct future

efforts towards new therapeutic approaches that will target specific molecular alterations in the tumor cell.

6. References

- Abe, M., Ohira, M., Kaneda, A., Yagi, Y., Yamamoto, S., Kitano, Y., Takato, T., Nakagawara, A. & Ushijima, T. (2005). CpG island methylator phenotype is a strong determinant of poor prognosis in neuroblastomas. *Cancer Res* 65(3): 828-34.
- Abe, M., Westermann, F., Nakagawara, A., Takato, T., Schwab, M. & Ushijima, T. (2007). Marked and independent prognostic significance of the CpG island methylator phenotype in neuroblastomas. *Cancer Lett* 247(2): 253-8.
- Agathangelou, A., Dallol, A., Zochbauer-Muller, S., Morrissey, C., Honorio, S., Hesson, L., Martinsson, T., Fong, K. M., Kuo, M. J., Yuen, P. W., Maher, E. R., Minna, J. D. & Latif, F. (2003). Epigenetic inactivation of the candidate 3p21.3 suppressor gene BLU in human cancers. *Oncogene* 22(10): 1580-8.
- Alaminos, M., Davalos, V., Cheung, N. K., Gerald, W. L. & Esteller, M. (2004). Clustering of gene hypermethylation associated with clinical risk groups in neuroblastoma. *J Natl Cancer Inst* 96(16): 1208-19.
- Ambros, P. F., Ambros, I. M., Brodeur, G. M., Haber, M., Khan, J., Nakagawara, A., Schleiermacher, G., Speleman, F., Spitz, R., London, W. B., Cohn, S. L., Pearson, A. D. & Maris, J. M. (2009). International consensus for neuroblastoma molecular diagnostics: report from the International Neuroblastoma Risk Group (INRG) Biology Committee. *Br J Cancer* 100(9): 1471-82.
- Amiel, J., Laudier, B., Attie-Bitach, T., Trang, H., de Pontual, L., Gener, B., Trochet, D., Etchevers, H., Ray, P., Simonneau, M., Vekemans, M., Munnich, A., Gaultier, C. & Lyonnet, S. (2003). Polyalanine expansion and frameshift mutations of the paired-like homeobox gene PHOX2B in congenital central hypoventilation syndrome. *Nat Genet* 33(4): 459-461.
- Astuti, D., Agathangelou, A., Honorio, S., Dallol, A., Martinsson, T., Kogner, P., Cummins, C., Neumann, H. P., Voutilainen, R., Dahia, P., Eng, C., Maher, E. R. & Latif, F. (2001). RASSF1A promoter region CpG island hypermethylation in pheochromocytomas and neuroblastoma tumours. *Oncogene* 20(51): 7573-7.
- Attiyeh, E. F., London, W. B., Mosse, Y. P., Wang, Q., Winter, C., Khazi, D., McGrady, P. W., Seeger, R. C., Look, A. T., Shimada, H., Brodeur, G. M., Cohn, S. L., Matthay, K. K. & Maris, J. M. (2005). Chromosome 1p and 11q deletions and outcome in neuroblastoma. *N Engl J Med* 353(21): 2243-53.
- Baade, P. D., Youlten, D. R., Valery, P. C., Hassall, T., Ward, L., Green, A. C. & Aitken, J. F. (2010). Population-based survival estimates for childhood cancer in Australia during the period 1997-2006. *Br J Cancer* 103(11): 1663-70.
- Bachetti, T., Di Paolo, D., Di Lascio, S., Mirisola, V., Brignole, C., Bellotti, M., Caffa, I., Ferraris, C., Fiore, M., Fornasari, D., Chiarle, R., Borghini, S., Pfeffer, U., Ponzoni, M., Ceccherini, I. & Perri, P. (2010). PHOX2B-mediated regulation of ALK expression: in vitro identification of a functional relationship between two genes involved in neuroblastoma. *PLoS One* 5(10).
- Bader, S. A., Fasching, C., Brodeur, G. M. & Stanbridge, E. J. (1991). Dissociation of suppression of tumorigenicity and differentiation in vitro effected by transfer of

- single human chromosomes into human neuroblastoma cells. *Cell Growth Differ* 2(5): 245-55.
- Banelli, B., Bonassi, S., Casciano, I., Mazzocco, K., Di Vinci, A., Scaruffi, P., Brigati, C., Allemanni, G., Borzi, L., Tonini, G. P. & Romani, M. (2010). Outcome prediction and risk assessment by quantitative pyrosequencing methylation analysis of the SFN gene in advanced stage, high-risk, neuroblastic tumor patients. *Int J Cancer* 126(3): 656-68.
- Banelli, B., Casciano, I., Croce, M., Di Vinci, A., Gelvi, I., Pagnan, G., Brignole, C., Allemanni, G., Ferrini, S., Ponzoni, M. & Romani, M. (2002). Expression and methylation of CASP8 in neuroblastoma: identification of a promoter region. *Nat Med* 8(12): 1333-5; author reply 1335.
- Banelli, B., Di Vinci, A., Gelvi, I., Casciano, I., Allemanni, G., Bonassi, S. & Romani, M. (2005a). DNA methylation in neuroblastic tumors. *Cancer Lett* 228(1-2): 37-41.
- Banelli, B., Gelvi, I., Di Vinci, A., Scaruffi, P., Casciano, I., Allemanni, G., Bonassi, S., Tonini, G. P. & Romani, M. (2005b). Distinct CpG methylation profiles characterize different clinical groups of neuroblastic tumors. *Oncogene* 24(36): 5619-28.
- Baylin, S. B. (2005). DNA methylation and gene silencing in cancer. *Nat Clin Pract Oncol* 2 Suppl 1: S4-11.
- Bown, N., Cotterill, S., Lastowska, M., O'Neill, S., Pearson, A. D., Plantaz, D., Meddeb, M., Danglot, G., Brinkschmidt, C., Christiansen, H., Laureys, G., Speleman, F., Nicholson, J., Bernheim, A., Betts, D. R., Vandesompele, J. & Van Roy, N. (1999). Gain of chromosome arm 17q and adverse outcome in patients with neuroblastoma. *N Engl J Med* 340(25): 1954-61.
- Brodeur, G. M. & Maris, J. M. (2006). Neuroblastoma. *Principles and Practice of Pediatric Oncology*. P. A. Pizzo & D. G. Poplack, Lippincott Williams & Wilkins: 933-970.
- Brodeur, G. M. & Seeger, R. C. (1986). Gene amplification in human neuroblastomas: basic mechanisms and clinical implications. *Cancer Genet Cytogenet* 19(1-2): 101-11.
- Buckley, P. G., Das, S., Bryan, K., Watters, K. M., Alcock, L., Koster, J., Versteeg, R. & Stallings, R. L. (2011). Genome-wide DNA methylation analysis of neuroblastic tumors reveals clinically relevant epigenetic events and large-scale epigenomic alterations localized to telomeric regions. *Int J Cancer* 128(10): 2296-305.
- Burbee, D. G., Forgacs, E., Zöchbauer-Müller, S., Shivakumar, L., Fong, K., Gao, B., Randle, D., Kondo, M., Virmani, A., Bader, S., Sekido, Y., Latif, F., Milchgrub, S., Toyooka, S., Gazdar, A. F., Lerman, M. I., Zbarovsky, E., White, M. & Minna, J. D. (2001). Epigenetic Inactivation of RASSF1A in Lung and Breast Cancers and Malignant Phenotype Suppression. *Journal of the National Cancer Institute* 93(9): 691-699.
- Calvo, R., West, J., Franklin, W., Erickson, P., Bemis, L., Li, E., Helfrich, B., Bunn, P., Roche, J., Brambilla, E., Rosell, R., Gemmill, R. M. & Drabkin, H. A. (2000). Altered HOX and WNT7A expression in human lung cancer. *Proc Natl Acad Sci U S A* 97(23): 12776-81.
- Capasso, M., Devoto, M., Hou, C., Asgharzadeh, S., Glessner, J. T., Attiyeh, E. F., Mosse, Y. P., Kim, C., Diskin, S. J., Cole, K. A., Bosse, K., Diamond, M., Laudenslager, M., Winter, C., Bradfield, J. P., Scott, R. H., Jagannathan, J., Garriss, M., McConville, C., London, W. B., Seeger, R. C., Grant, S. F., Li, H., Rahman, N., Rappaport, E., Hakonarson, H. & Maris, J. M. (2009). Common variations in BARD1 influence susceptibility to high-risk neuroblastoma. *Nat Genet* 41(6): 718-723.

- Capasso, M. & Diskin, S. J. (2010). Genetics and genomics of neuroblastoma. *Cancer Treat Res* 155: 65-84.
- Caren, H., Abel, F., Kogner, P. & Martinsson, T. (2008). High incidence of DNA mutations and gene amplifications of the ALK gene in advanced sporadic neuroblastoma tumours. *Biochem J* 416(2): 153-9.
- Caren, H., Fransson, S., Ejekkar, K., Kogner, P. & Martinsson, T. (2007). Genetic and epigenetic changes in the common 1p36 deletion in neuroblastoma tumours. *Br J Cancer* 97(10): 1416-24.
- Caron, H., van Sluis, P., Buschman, R., Pereira do Tanque, R., Maes, P., Beks, L., de Kraker, J., Voute, P. A., Vergnaud, G., Westerveld, A., Slater, R. & Versteeg, R. (1996a). Allelic loss of the short arm of chromosome 4 in neuroblastoma suggests a novel tumour suppressor gene locus. *Hum Genet* 97(6): 834-7.
- Caron, H., van Sluis, P., de Kraker, J., Bökkerink, J., Egeler, M., Laureys, G., Slater, R., Westerveld, A., Voûte, P. A. & Versteeg, R. (1996b). Allelic Loss of Chromosome 1p as a Predictor of Unfavorable Outcome in Patients with Neuroblastoma. *New England Journal of Medicine* 334(4): 225-230.
- Casciano, I., Banelli, B., Croce, M., De Ambrosis, A., di Vinci, A., Gelvi, I., Pagnan, G., Brignole, C., Allemanni, G., Ferrini, S., Ponzoni, M. & Romani, M. (2004). Caspase-8 gene expression in neuroblastoma. *Ann N Y Acad Sci* 1028: 157-67.
- Chen, Y., Takita, J., Choi, Y. L., Kato, M., Ohira, M., Sanada, M., Wang, L., Soda, M., Kikuchi, A., Igarashi, T., Nakagawara, A., Hayashi, Y., Mano, H. & Ogawa, S. (2008). Oncogenic mutations of ALK kinase in neuroblastoma. *Nature* 455(7215): 971-4.
- Cohn, S. L., Pearson, A. D., London, W. B., Monclair, T., Ambros, P. F., Brodeur, G. M., Faldut, A., Hero, B., Iehara, T., Machin, D., Mosseri, V., Simon, T., Garaventa, A., Castel, V. & Matthay, K. K. (2009). The International Neuroblastoma Risk Group (INRG) classification system: an INRG Task Force report. *J Clin Oncol* 27(2): 289-97.
- Curtis, D. J. & McCormack, M. P. (2010). The Molecular Basis of Lmo2-Induced T-Cell Acute Lymphoblastic Leukemia. *Clinical Cancer Research* 16(23): 5618-5623.
- Das, P. M. & Singal, R. (2004). DNA methylation and cancer. *J Clin Oncol* 22(22): 4632-42.
- De Brouwer, S., De Preter, K., Kumps, C., Zabrocki, P., Porcu, M., Westerhout, E. M., Lakeman, A., Vandesompele, J., Hoebeeck, J., Van Maerken, T., De Paepe, A., Laureys, G., Schulte, J. H., Schramm, A., Van Den Broecke, C., Vermeulen, J., Van Roy, N., Beiske, K., Renard, M., Noguera, R., Delattre, O., Janoueix-Lerosey, I., Kogner, P., Martinsson, T., Nakagawara, A., Ohira, M., Caron, H., Eggert, A., Cools, J., Versteeg, R. & Speleman, F. (2010). Meta-analysis of neuroblastomas reveals a skewed ALK mutation spectrum in tumors with MYCN amplification. *Clin Cancer Res* 16(17): 4353-62.
- Diskin, S. J., Hou, C., Glessner, J. T., Attiyeh, E. F., Laudenslager, M., Bosse, K., Cole, K., Mosse, Y. P., Wood, A., Lynch, J. E., Pecor, K., Diamond, M., Winter, C., Wang, K., Kim, C., Geiger, E. A., McGrady, P. W., Blakemore, A. I., London, W. B., Shaikh, T. H., Bradfield, J., Grant, S. F., Li, H., Devoto, M., Rappaport, E. R., Hakonarson, H. & Maris, J. M. (2009). Copy number variation at 1q21.1 associated with neuroblastoma. *Nature* 459(7249): 987-91.
- Ejekkar, K., Aburatani, H., Abrahamsson, J., Kogner, P. & Martinsson, T. (1998). Loss of heterozygosity of 3p markers in neuroblastoma tumours implicate a tumour-suppressor locus distal to the FHIT gene. *Br J Cancer* 77(11): 1787-91.

- Esteller, M. & Herman, J. G. (2002). Cancer as an epigenetic disease: DNA methylation and chromatin alterations in human tumours. *J Pathol* 196(1): 1-7.
- Foley, D. L., Craig, J. M., Morley, R., Olsson, C. A., Dwyer, T., Smith, K. & Saffery, R. (2009). Prospects for epigenetic epidemiology. *Am J Epidemiol* 169(4): 389-400.
- Fong, C. T., White, P. S., Peterson, K., Sapienza, C., Cavenee, W. K., Kern, S. E., Vogelstein, B., Cantor, A. B., Look, A. T. & Brodeur, G. M. (1992). Loss of heterozygosity for chromosomes 1 or 14 defines subsets of advanced neuroblastomas. *Cancer Res* 52(7): 1780-5.
- Freeman, J. L., Perry, G. H., Feuk, L., Redon, R., McCarroll, S. A., Altshuler, D. M., Aburatani, H., Jones, K. W., Tyler-Smith, C., Hurles, M. E., Carter, N. P., Scherer, S. W. & Lee, C. (2006). Copy number variation: new insights in genome diversity. *Genome Res* 16(8): 949-61.
- George, R. E., Attiyeh, E. F., Li, S., Moreau, L. A., Neuberg, D., Li, C., Fox, E. A., Meyerson, M., Diller, L., Fortina, P., Look, A. T. & Maris, J. M. (2007). Genome-wide analysis of neuroblastomas using high-density single nucleotide polymorphism arrays. *PLoS One* 2(2): e255.
- George, R. E., Lahti, J. M., Adamson, P. C., Zhu, K., Finkelstein, D., Ingle, A. M., Reid, J. M., Krailo, M., Neuberg, D., Blaney, S. M. & Diller, L. (2010). Phase I study of decitabine with doxorubicin and cyclophosphamide in children with neuroblastoma and other solid tumors: a Children's Oncology Group study. *Pediatr Blood Cancer* 55(4): 629-38.
- George, R. E., Sanda, T., Hanna, M., Frohling, S., Luther, W., 2nd, Zhang, J., Ahn, Y., Zhou, W., London, W. B., McGrady, P., Xue, L., Zozulya, S., Gregor, V. E., Webb, T. R., Gray, N. S., Gilliland, D. G., Diller, L., Greulich, H., Morris, S. W., Meyerson, M. & Look, A. T. (2008). Activating mutations in ALK provide a therapeutic target in neuroblastoma. *Nature* 455(7215): 975-8.
- Gilbert, P. M., Mouw, J. K., Unger, M. A., Lakins, J. N., Gbegnon, M. K., Clemmer, V. B., Benezra, M., Licht, J. D., Boudreau, N. J., Tsai, K. K., Welm, A. L., Feldman, M. D., Weber, B. L. & Weaver, V. M. (2010). HOXA9 regulates BRCA1 expression to modulate human breast tumor phenotype. *J Clin Invest* 120(5): 1535-50.
- Goffin, J. & Eisenhauer, E. (2002). DNA methyltransferase inhibitors-state of the art. *Ann Oncol* 13(11): 1699-716.
- Gonzalez-Gomez, P., Bello, M. J., Lomas, J., Arjona, D., Alonso, M. E., Aminosos, C., Lopez-Marin, I., Anselmo, N. P., Sarasa, J. L., Gutierrez, M., Casartelli, C. & Rey, J. A. (2003). Aberrant methylation of multiple genes in neuroblastic tumours. relationship with MYCN amplification and allelic status at 1p. *Eur J Cancer* 39(10): 1478-85.
- Grau, E., Martinez, F., Orellana, C., Canete, A., Yanez, Y., Oltra, S., Noguera, R., Hernandez, M., Bermudez, J. D. & Castel, V. (2010). Hypermethylation of apoptotic genes as independent prognostic factor in neuroblastoma disease. *Mol Carcinog* 50(3): 153-62.
- Hamrick, S. E., Olshan, A. F., Neglia, J. P. & Pollock, B. H. (2001). Association of pregnancy history and birth characteristics with neuroblastoma: a report from the Children's Cancer Group and the Pediatric Oncology Group. *Paediatr Perinat Epidemiol* 15(4): 328-37.
- Harada, K., Toyooka, S., Maitra, A., Maruyama, R., Toyooka, K. O., Timmons, C. F., Tomlinson, G. E., Mastrangelo, D., Hay, R. J., Minna, J. D. & Gazdar, A. F. (2002).

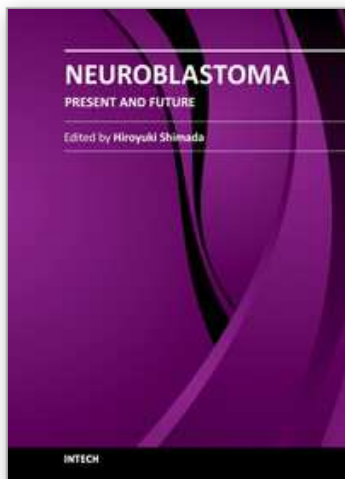
- Aberrant promoter methylation and silencing of the RASSF1A gene in pediatric tumors and cell lines. *Oncogene* 21(27): 4345-9.
- Hausmann, G., O'Reilly, L. A., van Driel, R., Beaumont, J. G., Strasser, A., Adams, J. M. & Huang, D. C. (2000). Pro-apoptotic apoptosis protease-activating factor 1 (Apaf-1) has a cytoplasmic localization distinct from Bcl-2 or Bcl-x(L). *J Cell Biol* 149(3): 623-34.
- Hermeking, H. (2003). The 14-3-3 cancer connection. *Nat Rev Cancer* 3(12): 931-943.
- Hero, B., Simon, T., Spitz, R., Ernestus, K., Gnekow, A. K., Scheel-Walter, H. G., Schwabe, D., Schilling, F. H., Benz-Bohm, G. & Berthold, F. (2008). Localized infant neuroblastomas often show spontaneous regression: results of the prospective trials NB95-S and NB97. *J Clin Oncol* 26(9): 1504-10.
- Hoebeeck, J., Michels, E., Pattyn, F., Combaret, V., Vermeulen, J., Yigit, N., Hoyoux, C., Laureys, G., De Paepe, A., Speleman, F. & Vandesompele, J. (2009). Aberrant methylation of candidate tumor suppressor genes in neuroblastoma. *Cancer Lett* 273(2): 336-46.
- Islam, A., Kageyama, H., Takada, N., Kawamoto, T., Takayasu, H., Isogai, E., Ohira, M., Hashizume, K., Kobayashi, H., Kaneko, Y. & Nakagawara, A. (2000). High expression of Survivin, mapped to 17q25, is significantly associated with poor prognostic factors and promotes cell survival in human neuroblastoma. *Oncogene* 19(5): 617-23.
- Janoueix-Lerosey, I., Lequin, D., Brugieres, L., Ribeiro, A., de Pontual, L., Combaret, V., Raynal, V., Puisieux, A., Schleiermacher, G., Pierron, G., Valteau-Couanet, D., Frebourg, T., Michon, J., Lyonnet, S., Amiel, J. & Delattre, O. (2008). Somatic and germline activating mutations of the ALK kinase receptor in neuroblastoma. *Nature* 455(7215): 967-70.
- Janoueix-Lerosey, I., Schleiermacher, G., Michels, E., Mosseri, V., Ribeiro, A., Lequin, D., Vermeulen, J., Couturier, J., Peuchmaur, M., Valent, A., Plantaz, D., Rubie, H., Valteau-Couanet, D., Thomas, C., Combaret, V., Rousseau, R., Eggert, A., Michon, J., Speleman, F. & Delattre, O. (2009). Overall genomic pattern is a predictor of outcome in neuroblastoma. *J Clin Oncol* 27(7): 1026-33.
- Jones, P. A. & Baylin, S. B. (2002). The fundamental role of epigenetic events in cancer. *Nat Rev Genet* 3(6): 415-28.
- Kaneko, Y., Kanda, N., Maseki, N., Sakurai, M., Tsuchida, Y., Takeda, T., Okabe, I. & Sakurai, M. (1987). Different Karyotypic Patterns in Early and Advanced Stage Neuroblastomas. *Cancer Research* 47(1): 311-318.
- Kulis, M. & Esteller, M. (2010). DNA methylation and cancer. *Adv Genet* 70: 27-56.
- Lastowska, M., Cullinane, C., Variend, S., Cotterill, S., Bown, N., O'Neill, S., Mazzocco, K., Roberts, P., Nicholson, J., Ellershaw, C., Pearson, A. D. & Jackson, M. S. (2001). Comprehensive genetic and histopathologic study reveals three types of neuroblastoma tumors. *J Clin Oncol* 19(12): 3080-90.
- Lazcoz, P., Munoz, J., Nistal, M., Pestana, A., Encio, I. & Castresana, J. S. (2006). Frequent promoter hypermethylation of RASSF1A and CASP8 in neuroblastoma. *BMC Cancer* 6: 254.
- Limpt, V. v., Schramm, A., Lakeman, A., Sluis, P. v., Chan, A., Noesel, M. v., Baas, F., Caron, H., Eggert, A. & Versteeg, R. (2004). The Phox2B homeobox gene is mutated in sporadic neuroblastomas. *Oncogene* 23(57): 9280-9288.

- London, W. B., Castleberry, R. P., Matthay, K. K., Look, A. T., Seeger, R. C., Shimada, H., Thorner, P., Brodeur, G., Maris, J. M., Reynolds, C. P. & Cohn, S. L. (2005). Evidence for an age cutoff greater than 365 days for neuroblastoma risk group stratification in the Children's Oncology Group. *J Clin Oncol* 23(27): 6459-65.
- Look, A. T., Hayes, F. A., Shuster, J. J., Douglass, E. C., Castleberry, R. P., Bowman, L. C., Smith, E. I. & Brodeur, G. M. (1991). Clinical relevance of tumor cell ploidy and N-myc gene amplification in childhood neuroblastoma: a Pediatric Oncology Group study. *J Clin Oncol* 9(4): 581-91.
- Mack, G. S. (2006). Epigenetic cancer therapy makes headway. *J Natl Cancer Inst* 98(20): 1443-4.
- Margetts, C. D. E., Morris, M., Astuti, D., Gentle, D. C., Cascon, A., McDonald, F. E., Catchpoole, D., Robledo, M., Neumann, H. P. H., Latif, F. & Maher, E. R. (2008). Evaluation of a functional epigenetic approach to identify promoter region methylation in pheochromocytoma and neuroblastoma. *Endocrine-Related Cancer* 15(3): 777-786.
- Maris, J. M., Chatten, J., Meadows, A. T., Biegel, J. A. & Brodeur, G. M. (1997). Familial neuroblastoma: a three-generation pedigree and a further association with Hirschsprung disease. *Med Pediatr Oncol* 28(1): 1-5.
- Maris, J. M., Hogarty, M. D., Bagatell, R. & Cohn, S. L. (2007). Neuroblastoma. *Lancet* 369(9579): 2106-20.
- Maris, J. M. & Matthay, K. K. (1999). Molecular biology of neuroblastoma. *J Clin Oncol* 17(7): 2264-79.
- Maris, J. M., Mosse, Y. P., Bradfield, J. P., Hou, C., Monni, S., Scott, R. H., Asgharzadeh, S., Attiyeh, E. F., Diskin, S. J., Laudenslager, M., Winter, C., Cole, K. A., Glessner, J. T., Kim, C., Frackelton, E. C., Casalunovo, T., Eckert, A. W., Capasso, M., Rappaport, E. F., McConville, C., London, W. B., Seeger, R. C., Rahman, N., Devoto, M., Grant, S. F., Li, H. & Hakonarson, H. (2008). Chromosome 6p22 locus associated with clinically aggressive neuroblastoma. *N Engl J Med* 358(24): 2585-93.
- Martinez, R., Schackert, G. & Esteller, M. (2007). Hypermethylation of the proapoptotic gene TMS1/ASC: prognostic importance in glioblastoma multiforme. *J Neurooncol* 82(2): 133-9.
- Martinsson, T., Eriksson, T., Abrahamsson, J., Caren, H., Hansson, M., Kogner, P., Kamaraj, S., Schonherr, C., Weinmar, J., Ruuth, K., Palmer, R. H. & Hallberg, B. (2011). Appearance of the novel activating F1174S ALK mutation in neuroblastoma correlates with aggressive tumor progression and unresponsiveness to therapy. *Cancer Res* 71(1): 98-105.
- Michalowski, M. B., de Fraipont, F., Plantaz, D., Michelland, S., Combaret, V. & Favrot, M. C. (2008). Methylation of tumor-suppressor genes in neuroblastoma: The RASSF1A gene is almost always methylated in primary tumors. *Pediatr Blood Cancer* 50(1): 29-32.
- Misawa, A., Tanaka, S., Yagyu, S., Tsuchiya, K., Iehara, T., Sugimoto, T. & Hosoi, H. (2009). RASSF1A hypermethylation in pretreatment serum DNA of neuroblastoma patients: a prognostic marker. *Br J Cancer* 100(2): 399-404.
- Mosse, Y. P., Laudenslager, M., Khazi, D., Carlisle, A. J., Winter, C. L., Rappaport, E. & Maris, J. M. (2004). Germline PHOX2B mutation in hereditary neuroblastoma. *Am J Hum Genet* 75(4): 727-30.

- Mosse, Y. P., Laudenslager, M., Longo, L., Cole, K. A., Wood, A., Attiyeh, E. F., Laquaglia, M. J., Sennett, R., Lynch, J. E., Perri, P., Laureys, G., Speleman, F., Kim, C., Hou, C., Hakonarson, H., Torkamani, A., Schork, N. J., Brodeur, G. M., Tonini, G. P., Rappaport, E., Devoto, M. & Maris, J. M. (2008). Identification of ALK as a major familial neuroblastoma predisposition gene. *Nature* 455(7215): 930-5.
- Mosse, Y. P., Wood, A. & Maris, J. M. (2009). Inhibition of ALK signaling for cancer therapy. *Clin Cancer Res* 15(18): 5609-14.
- Munzer, C., Menegaux, F., Lacour, B., Valteau-Couanet, D., Michon, J., Coze, C., Bergeron, C., Auvrignon, A., Bernard, F., Thomas, C., Vannier, J. P., Kanold, J., Rubie, H., Hemon, D. & Clavel, J. (2008). Birth-related characteristics, congenital malformation, maternal reproductive history and neuroblastoma: the ESCALE study (SFCE). *Int J Cancer* 122(10): 2315-21.
- The NCBI handbook* (2002). [Internet] Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information.
- Nguyễn, L. B., Diskin, S. J., Capasso, M., Wang, K., Diamond, M. A., Glessner, J., Kim, C., Attiyeh, E. F., Mosse, Y. P., Cole, K., Iolascon, A., Devoto, M., Hakonarson, H., Li, H. K. & Maris, J. M. (2011). Phenotype Restricted Genome-Wide Association Study Using a Gene-Centric Approach Identifies Three Low-Risk Neuroblastoma Susceptibility Loci. *PLoS genetics* 7(3): e1002026.
- Okawa, E. R., Gotoh, T., Manne, J., Igarashi, J., Fujita, T., Silverman, K. A., Xhao, H., Mosse, Y. P., White, P. S. & Brodeur, G. M. (2008). Expression and sequence analysis of candidates for the 1p36.31 tumor suppressor gene deleted in neuroblastomas. *Oncogene* 27(6): 803-10.
- Oue, T., Fukuzawa, M., Kusafuka, T., Kohmoto, Y., Imura, K., Nagahara, S. & Okada, A. (1996). In situ detection of DNA fragmentation and expression of bcl-2 in human neuroblastoma: relation to apoptosis and spontaneous regression. *J Pediatr Surg* 31(2): 251-7.
- Passoni, L., Longo, L., Collini, P., Coluccia, A. M., Bozzi, F., Podda, M., Gregorio, A., Gambini, C., Garaventa, A., Pistoia, V., Del Grosso, F., Tonini, G. P., Cheng, M., Gambacorti-Passerini, C., Anichini, A., Fossati-Bellani, F., Di Nicola, M. & Luksch, R. (2009). Mutation-independent anaplastic lymphoma kinase overexpression in poor prognosis neuroblastoma patients. *Cancer Res* 69(18): 7338-46.
- Plantaz, D., Mohapatra, G., Matthay, K. K., Pellarin, M., Seeger, R. C. & Feuerstein, B. G. (1997). Gain of chromosome 17 is the most frequent abnormality detected in neuroblastoma by comparative genomic hybridization. *Am J Pathol* 150(1): 81-9.
- Prendergast, G. & Ziff, E. (1991). Methylation-sensitive sequence-specific DNA binding by the c-Myc basic region. *Science* 251(4990): 186-189.
- Qiu, G.-H., Tan, L. K. S., Loh, K. S., Lim, C. Y., Srivastava, G., Tsai, S.-T., Tsao, S. W. & Tao, Q. (2004). The candidate tumor suppressor gene BLU, located at the commonly deleted region 3p21.3, is an E2F-regulated, stress-responsive gene and inactivated by both epigenetic and genetic mechanisms in nasopharyngeal carcinoma. *Oncogene* 23(27): 4793-4806.
- Raabe, E. H., Laudenslager, M., Winter, C., Wasserman, N., Cole, K., LaQuaglia, M., Maris, D. J., Mosse, Y. P. & Maris, J. M. (2008). Prevalence and functional consequence of PHOX2B mutations in neuroblastoma. *Oncogene* 27(4): 469-476.
- Ries, L.A.G., Smith, M.A., Gurney, J.G., Linet, M., Tamra, T., Young, J. L. & Bunin, G. R. (1999). Cancer Incidence and Survival among Children and Adolescents: United

- States SEER Program 1975-1995. National Cancer Institute, SEER Program. Bethesda, MD. NIH Pub. No. 99-4649: 17-34.
- Roberts, D. D. (1996). Regulation of tumor growth and metastasis by thrombospondin-1. *FASEB J* 10(10): 1183-91.
- Rodenhiser, D. & Mann, M. (2006). Epigenetics and human disease: translating basic biology into clinical applications. *CMAJ* 174(3): 341-8.
- Ross, R. A. & Spengler, B. A. (2004). The conundrum posed by cellular heterogeneity in analysis of human neuroblastoma. *J Natl Cancer Inst* 96(16): 1192-3.
- Schleiermacher, G., Janoueix-Lerosey, I., Ribeiro, A., Klijanienko, J., Couturier, J., Pierron, G., Mosseri, V., Valent, A., Auger, N., Plantaz, D., Rubie, H., Valteau-Couanet, D., Bourdeaut, F., Combaret, V., Bergeron, C., Michon, J. & Delattre, O. (2010). Accumulation of segmental alterations determines progression in neuroblastoma. *J Clin Oncol* 28(19): 3122-30.
- Schwab, M., Westermann, F., Hero, B. & Berthold, F. (2003). Neuroblastoma: biology and molecular and chromosomal pathology. *Lancet Oncol* 4(8): 472-80.
- Seeger, R. C., Wada, R., Brodeur, G. M., Moss, T. J., Bjork, R. L., Sousa, L. & Slamon, D. J. (1988). Expression of N-myc by neuroblastomas with one or multiple copies of the oncogene. *Prog Clin Biol Res* 271: 41-9.
- Takita, J., Ishii, M., Tsutsumi, S., Tanaka, Y., Kato, K., Toyoda, Y., Hanada, R., Yamamoto, K., Hayashi, Y. & Aburatani, H. (2004). Gene expression profiling and identification of novel prognostic marker genes in neuroblastoma. *Genes Chromosomes Cancer* 40(2): 120-32.
- Tanaka, T., Sugimoto, T. & Sawada, T. (1998). Prognostic discrimination among neuroblastomas according to Ha-ras/trk A gene expression: a comparison of the profiles of neuroblastomas detected clinically and those detected through mass screening. *Cancer* 83(8): 1626-33.
- Teitz, T., Wei, T., Liu, D., Valentine, V., Valentine, M., Grenet, J., Lahti, J. M. & Kidd, V. J. (2002). Caspase-9 and Apaf-1 are expressed and functionally active in human neuroblastoma tumor cell lines with 1p36 LOH and amplified MYCN. *Oncogene* 21(12): 1848-58.
- Teitz, T., Wei, T., Valentine, M. B., Vanin, E. F., Grenet, J., Valentine, V. a., Behm, F. G., Look, A. T., Lahti, J. M. & Kidd, V. J. (2000). Caspase 8 is deleted or silenced preferentially in childhood neuroblastomas with amplification of MYCN. *Nat Med* 6: 529-535.
- The American Association for Cancer Research Human Epigenome Task Force; The European Union Network of Excellence Scientific Advisory Board (2008). Moving AEAD with an international human epigenome project. *Nature* 454: 711-715.
- Trochet, D., Bourdeaut, F., Janoueix-Lerosey, I., Deville, A., de Pontual, L., Schleiermacher, G., Coze, C., Philip, N., Frebourg, T., Munnich, A., Lyonnet, S., Delattre, O. & Amiel, J. (2004). Germline mutations of the paired-like homeobox 2B (PHOX2B) gene in neuroblastoma. *Am J Hum Genet* 74(4): 761-4.
- Urayama, K. Y., Von Behren, J. & Reynolds, P. (2007). Birth characteristics and risk of neuroblastoma in young children. *Am J Epidemiol* 165(5): 486-95.
- van Noesel, M. M., van Bezouw, S., Salomons, G. S., Voute, P. A., Pieters, R., Baylin, S. B., Herman, J. G. & Versteeg, R. (2002). Tumor-specific down-regulation of the tumor necrosis factor-related apoptosis-inducing ligand decoy receptors DcR1 and DcR2 is associated with dense promoter hypermethylation. *Cancer Res* 62(7): 2157-61.

- van Noesel, M. M. & Versteeg, R. (2004). Pediatric neuroblastomas: genetic and epigenetic 'danse macabre'. *Gene* 325: 1-15.
- Vandesompele, J., Van Roy, N., Van Gele, M., Laureys, G., Ambros, P., Heimann, P., Devalck, C., Schuurin, E., Brock, P., Otten, J., Gyselinck, J., De Paepe, A. & Speleman, F. (1998). Genetic heterogeneity of neuroblastoma studied by comparative genomic hybridization. *Genes Chromosomes Cancer* 23(2): 141-52.
- Wang, K., Diskin, S. J., Zhang, H., Attiyeh, E. F., Winter, C., Hou, C., Schnepp, R. W., Diamond, M., Bosse, K., Mayes, P. A., Glessner, J., Kim, C., Frackelton, E., Garriss, M., Wang, Q., Glaberson, W., Chiavacci, R., Nguyen, L., Jagannathan, J., Saeki, N., Sasaki, H., Grant, S. F., Iolascon, A., Mosse, Y. P., Cole, K. A., Li, H., Devoto, M., McGrady, P. W., London, W. B., Capasso, M., Rahman, N., Hakonarson, H. & Maris, J. M. (2011). Integrative genomics identifies LMO1 as a neuroblastoma oncogene. *Nature* 469(7329): 216-20.
- Weese-Mayer, D. E., Berry-Kravis, E. M. & Marazita, M. L. (2005). In pursuit (and discovery) of a genetic basis for congenital central hypoventilation syndrome. *Respir Physiol Neurobiol* 149(1-3): 73-82.
- Wellmann, A., Doseeva, V., Butscher, W., Raffeld, M., Fukushima, P., Stetler-Stevenson, M. & Gardner, K. (1997). The activated anaplastic lymphoma kinase increases cellular proliferation and oncogene up-regulation in rat 1a fibroblasts. *FASEB J* 11(12): 965-72.
- White, P. S., Thompson, P. M., Gotoh, T., Okawa, E. R., Igarashi, J., Kok, M., Winter, C., Gregory, S. G., Hogarty, M. D., Maris, J. M. & Brodeur, G. M. (2005). Definition and characterization of a region of 1p36.3 consistently deleted in neuroblastoma. *Oncogene* 24(16): 2684-94.
- Wong, I. H., Chan, J., Wong, J. & Tam, P. K. (2004). Ubiquitous aberrant RASSF1A promoter methylation in childhood neoplasia. *Clin Cancer Res* 10(3): 994-1002.
- Yagyu, S., Gotoh, T., Iehara, T., Miyachi, M., Katsumi, Y., Tsubai-Shimizu, S., Kikuchi, K., Tamura, S., Tsuchiya, K., Imamura, T., Misawa-Furihata, A., Sugimoto, T., Sawada, T. & Hosoi, H. (2008). Circulating methylated-DCR2 gene in serum as an indicator of prognosis and therapeutic efficacy in patients with MYCN nonamplified neuroblastoma. *Clin Cancer Res* 14(21): 7011-9.
- Yang, Q., Kiernan, C. M., Tian, Y., Salwen, H. R., Chlenski, A., Brumback, B. A., London, W. B. & Cohn, S. L. (2007). Methylation of CASP8, DCR2, and HIN-1 in neuroblastoma is associated with poor outcome. *Clin Cancer Res* 13(11): 3191-7.
- Yang, Q., Zage, P., Kagan, D., Tian, Y., Seshadri, R., Salwen, H. R., Liu, S., Chlenski, A. & Cohn, S. L. (2004). Association of epigenetic inactivation of RASSF1A with poor outcome in human neuroblastoma. *Clin Cancer Res* 10(24): 8493-500.
- Yang, Q. W., Liu, S., Tian, Y., Salwen, H. R., Chlenski, A., Weinstein, J. & Cohn, S. L. (2003). Methylation-associated silencing of the thrombospondin-1 gene in human neuroblastoma. *Cancer Res* 63(19): 6299-310.
- Zhang, C., Li, H., Zhou, G., Zhang, Q., Zhang, T., Li, J., Zhang, J., Hou, J., Liew, C. T. & Yin, D. (2007). Transcriptional silencing of the TMS1/ASC tumour suppressor gene by an epigenetic mechanism in hepatocellular carcinoma cells. *J Pathol* 212(2): 134-42.



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Neuroblastoma, once called "enigmatic", due to "unpredictable" clinical behaviors, is composed of biologically diverse tumors. Molecular/genomic properties unique to the individual tumors closely link to the clinical outcomes of patients. Establishing risk stratification models after analyzing biologic characteristics of each case has made a great success in patient management. However, the trend of improving survival rates in neuroblastoma over the last 30 years has started to level off, and currently available treatment modalities have almost reached to their maximized intensity. Furthermore, aggressive treatment causes significant long-term morbidities to the survivors. We really need to make the next step to the level of personalized medicine with more precise understanding of neuroblastoma biology. This book includes useful data and insights from the world's experts in this field. I believe this book can make an excellent contribution to all the investigators working hard and fighting for the children stricken by this disease.

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