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Neuroblastoma: Molecular Pathogenesis and Therapy

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Abstract

Neuroblastoma is a developmental tumor of young children arising from the embryonic sympathoadrenal lineage of the neural crest. Currently neuroblastoma is the primary cause of death from pediatric cancer for children between the age of 1 and 5 years and accounts for approximately 13% of all pediatric cancer mortality. Its clinical impact and its unique biology have made this aggressive malignancy the focus of a large concerted translational research effort. New insights into tumor biology are driving the development of new classification schemas; novel targeted therapeutic approaches include small molecule inhibitors, epigenetic, non-coding RNA, and cell-based immunologic therapies. Recent insights regarding the pathogenesis and biology of neuroblastoma will be placed in context with the current understanding of tumor biology and tumor/host interactions. Systematic classification of patients coupled with therapeutic advances point to a future of improved clinical outcomes for this biologically distinct and highly aggressive pediatric malignancy.

Keywords

Biology; epigenetics; epithelial-to-mesenchymal transition; neural crest development; oncogenes; review

Introduction

Neuroblastoma (NB) is the most common extra-cranial solid tumor in childhood. In 2010, the age-adjusted incidence in the United States was 10.7 cases per 1,000,000 persons aged 0–14 years (1). However, these numbers likely underestimate its true incidence as neuroblastoma regresses in some infants who therefore may never present to medical attention. The median age at the time of diagnosis is approximately 19 months and ranges from in-utero identification by fetal MRI (Figure 1) to the rare cases diagnosed each year in patients older than 19 years of age (2). Historically, neuroblastomas are slightly more common in boys and can be seen in all North American ethnic groups with 2010 incidence

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rates reported as 9.0 cases in Whites, 6.0 in Blacks, 6.3 in Asian/Pacific Islanders, 5 in American Indian/Alaska Native, and 6.5 in Hispanics per 1,000,000 children aged 0–19 (1).

Neuroblastoma remains distinct from other solid tumors due to its biological heterogeneity and range of clinical behavior spanning from spontaneous regression to cases of highly-aggressive metastatic disease unresponsive to standard and investigational anti-cancer treatment. Using historical overall and event-free survival rates combined with histological and biological criteria, patients with neuroblastoma can be assigned a pre-treatment risk classification (3). Although the histological and biologic characteristics used within classification schemas continue to evolve based on new scientific data, they are used to divide patients into low, intermediate and high-risk strata. In general, those with low risk disease have excellent event free and overall survival rates with observation only or minimal therapeutic interventions. The outcome of patients with intermediate risk disease, using primarily surgery and chemotherapy, have improved to the point where many groups are focused on using biological markers to help further decrease therapy in specific sub-populations of children (4). Patients with high-risk disease, comprising approximately half of all new neuroblastoma cases each year, require treatment with multi-modal therapy using induction chemotherapy, surgery, radiotherapy, high-dose chemotherapy with autologous stem cell rescue, biologic and immunotherapeutic maintenance therapy in order to improve their survival odds. Using this aggressive therapeutic strategy, the Children's Oncology Group (COG) has reported a 4-year event-free survival (EFS) of 59+5% in patients treated on the most recent Phase III clinical trial using ch14.18 immunotherapy (5). Therefore, as a significant number of patients will still relapse and eventually die of disease, it remains important for investigators to both better understand the origins of this disease and develop novel treatment strategies for those who are diagnosed with it.

Molecular Pathogenesis of Neuroblastoma – A Tumor of the Neural Crest

Neuroblastoma is a developmental malignancy arising within the neuronal ganglia of the peripheral sympathetic nervous system. These neuronal structures derive from the venterolateral neural crest cells, which migrate away from the neural tube early during embryogenesis (6). Thirty percent of neuroblastoma tumors arise within the adrenal medulla, approximately 60% will arise from abdominal paraspinal ganglia, and the remaining is from the sympathetic ganglia in the chest, head/neck and pelvis. As such, the clinical presentation and subsequent outcomes of neuroblastoma are highly variable. Long-term survival is primarily dependent on the degree of differentiation, with patients exhibiting more primitive crest-like tumors doing worse than patients with more differentiated tumors who have a more favorable outcome (7). The extensive clinical and pathologic heterogeneity of this malignancy reflects the unique developmental biology of the neural crest (8). Placing the pathogenesis of neuroblastoma in the context of neural crest embryogenesis may help to explain the complex molecular heterogeneity of this disease and help identify molecules and pathways for specific biologically-targeted interventions.

Sometimes referred to as the fourth germ layer, the neural crest is a transient embryologic tissue derived from neuroectoderm (9). In vertebrates during neural tube formation, a remarkable maturation process occurs within the neural crest, which responds to a complex

transcription factor/epigenetic regulatory schema (10, 11). Through this process, the earliest neural crest precursors *gain* multipotent differentiation potential and obtain a self-renewing phenotype reminiscent of embryonic stem cells. Subsequent cascading signaling gradients of BMP, Wnt, Notch and other ligands drive differentiation into epithelial, mesenchymal, and endothelial components of the face, trunk, and heart (12, 13) and include the peripheral sympathetic ganglia and neuroendocrine adrenal medulla (14). Inhibition of this maturation process may predispose early multipotent neural crest precursors to malignant transformation.

EMT and MET Transitions within the Neural Crest

A central component of neural crest maturation is a programmed epithelial-to-mesenchymal transition (EMT) (12, 15). During embryogenesis, a series of transcriptional factors including ZIC1, PAX3, TPAP2a, Notch and PRDM1A initiate the crest developmental pathway after the neural tube forms (16, 17). This distinguishes early neural crest cells from primitive neuroectoderm. Subsequent expression of the SOXE family (SOX 8, 9, 10) as well as ZEB2 and other factors, drive mesenchymal transformation (e.g. loss of E-Cadherins, loss of cell contacts, activation of metalloproteinases). Next, BMP, Wnt and FGF signaling within the microenvironment further drive differentiation of these mesenchymal migratory neural crest cells. The early neural crest is similar to other pluripotent cell populations -with regards to their self-renew capacity and ability to generate many different tissue types. Expression of pro-survival and pluripotency factors such as SOX10, FOXD3, C-Myc and MYCN allow these cells to become highly proliferative and resistant to apoptosis (18).

The observed clinical and pathological heterogeneity of neuroblastoma may well result from diverse molecular drivers disrupting this carefully orchestrated process at discrete stages of neural crest maturation (Figure 2). NB tumor initiating cells or cancer stem cells (CSCs) of various backgrounds may yield distinct tumor phenotypes according to the developmental stage of their crest precursors (19, 20). This concept is supported by the recent observation of tumorigenic stem cell-like subpopulations within neuroblastoma that differentially express elevated SOX10, E-Cadherin and other pre-migratory early crest markers (21). In addition, a distinct subset of highly undifferentiated neuroblastoma (Stage IVS or M4S) presents with metastatic disease in very young infants. Remarkably, some of these tumors spontaneously regress within months as the child matures, strongly suggesting that this subtype of NB requires non-cell autonomous growth factors for survival (22, 23). Alternatively, lesions arising from a more mesenchymal precursor may be highly metastatic and lack requirements for external growth factors. Controlled inhibition, but not mutation, of p53 is required for persistence of early crest precursors (24), which corresponds to the observation that NB is almost uniformly p53 wild-type at diagnosis yet resistant to apoptotic stresses (25, 26). Tumor initiating cells arising at later stages may yield more differentiated and therefore less malignant low stage tumors. Consideration of a uniquely dynamic and multipotent neural crest developmental program can guide the generation of novel and innovative therapeutics for crest derived malignancies such as neuroblastoma. Some of the well-defined oncogenic drivers of neuroblastoma are reviewed below.

Neuroblastoma Oncogenic Drivers and Transcriptional Networks

While the origins of neuroblastoma tumorigenesis arise from the disrupted development of neural crest precursors, no single genetic or epigenetic mutation has been found, after the DNA and RNA sequencing of over one thousand cases, to account for all cases of NB (27). Likewise, structural genomic changes have not been linked to NB tumorigenesis. For example, 1p deletion, MYCN amplification, or gain of 17q may identify subtypes of neuroblastoma and impact survival (28, 29), yet there is no common neuroblastoma-specific genomic alteration, LOH or genetic translocation uniformly ascribed to all high-risk neuroblastoma tumors. Thus, this extensive molecular heterogeneity supports the concept that neuroblastoma represents a spectrum of disease. Clinically, this presents a challenge as tumors that are phenotypically and morphologically very similar can have highly disparate responses to treatment. Consequently, extensive efforts have focused on characterizing the transcriptomes and oncogenic pathways active in the most aggressive and fatal subtypes (30–32). In addition to elucidating the genetic and epigenetic origins of neuroblastoma, these efforts are motivated by the potential to yield actionable therapeutic targets for this highly fatal cancer.

MYCN—The MYCN oncogene plays a major role in neuroblastoma tumorigenesis and defines an aggressive subset of tumors. Amplification of MYCN (defined as more than 10 copies) is found in about 20% of cases overall and confers a particularly poor prognosis. Well-defined transgenic mouse models confirm that deregulated MYCN expression targeted to the neural crest is sufficient to drive tumorigenesis with high penetrance (33, 34). This transcription factor both activates and represses genetic targets (e.g. mRNA, miRNAs, lncRNAs) through direct DNA binding as well as indirect protein/protein interaction mechanisms (35–38). Both MYCN and MYCC (C-MYC) have well described anti-p53, pro-proliferative functions and pro-EMT functions (31, 39). During normal embryogenesis and neural crest development, MYCN is transiently expressed in the ventral-lateral migrating crest cells destined to become sympathetic ganglia (40). Thus, it is not surprising to find high levels of MYCN in a subset of poorly differentiated aggressive neuroblastomas (7). This has translated to clinical approaches targeting MYCN and other downstream pathways such as MDM2 (RG3788, Roche Pharmaceuticals) (41, 42), ODC1 (difluoromethylornithine -DFMO) (43) and mTOR (Temazolamide) (44, 45). However, many high-risk cases have minimal MYCN expression, suggesting additional mechanisms for tumorigenesis independent of MYCN deregulation (46).

ALK—Activating mutations of ALK (anaplastic lymphoma kinase) are also implicated as oncogenic drivers of neuroblastoma (47). Mutations are found in almost all cases of familial neuroblastoma (<1% of total NB cases) and between 6–10% of spontaneous cases (48). This receptor tyrosine kinase (RTK) is also implicated as an oncogene in lymphomas and lung cancers where it is typically found as a translocated fusion gene (ALK-NPM) (49, 50). Recent studies link ALK to sympathetic neuron development and survival of migratory neural crest cells (51), as well as being essential for neurogenesis in Zebrafish models. This gene is an important regulator of stem cell functions, including STAT3 dependent self-renewal, and as a transcriptional target of MYCN, high expression predicts poor outcome (52). Recent data from genetically engineered mouse models of neuroblastoma confirm that

ALK and MYCN cooperate to promote tumorigenesis (53). Of note, this kinase is amenable to drug targeting, and potent ALK inhibitors are already in clinical trials for ALK mutant neuroblastoma.

PHOX2B—Germ line mutations of Paired-like Homeobox 2B (PHOX2B) are found in a subset of familial neuroblastoma and in about 4% of sporadic cases (54, 55). PHOX2B and PHOX2A drive differentiation of neural crest precursors toward sympathetic neurons (56). Mutations in this pathway are associated with neurocristopathies involving sympathetic and parasympathetic lineages such as Hirschsprung's disease and central hypoventilation syndrome (57, 58). Recently, PHOX2B loss-of-function mutations have been shown to block neuroblastoma differentiation by disrupting calcium regulation (59). PHOX2B may also inhibit ALK expression in neuroblastoma (60); further suggesting that loss of PHOX2B function contributes to the pathogenesis of a subset of neuroblastoma tumors.

Epigenetics—Chromatin immunoprecipitation with high throughput sequencing (ChIPseq) and RNA sequencing studies have demonstrated specific epigenetic patterns which distinguish neuroectoderm, neural crest, and more mature neural states (61). For example, crest-specific patterns of histone modifications (H3k27ac, H3K4me1 and p300) mark enhancers of genes such as SNAIL, SOX10 and FOXD3 involved in EMT (62). A subset of these crest-specific promoters share the 'bivalent' H3k27me3/ H3K4me3 marks of poised promoters associated with pluripotent ESCs. In addition, the major chromatin modifying complex SWI/SNF and its subunits BRG1, ARID1 and BRN play critical roles in crest maturation (63), and mutations of these factors are linked to neuroblastoma tumorigenesis as detailed below. DNMT3B dependent DNA demethylation also participates in neural crest maturation by activation of differentiation specific genetic pathways (64), and alterations in this methyltransferase promote tumorigenesis (65). The development of effective and specific epigenetic drugs may soon permit a reverse of pathogenic epigenetic modifications and therefore promote neuroblastoma to differentiate along its programmed neural crest maturation pathway.

ATRX is another epigenetic factor mutated in a distinct subtype of NB tumors presenting in older children and adolescents. Interestingly, this gene contains an ATPase/helicase domain and is another member of the SWI/SNF family of chromatin modifiers. Functionally, it is involved in regulation of telomere length (66). Mutations were found in 44% of Stage IV4 neuroblastoma cases presenting in children ≥12 years of age, but only 9% of cases in children <12 years of age. Notably, no mutations were identified in children less than 1 year of age (67). Older children with this mutation typically have slow growing, yet inexorably progressive forms of neuroblastoma with a high overall mortality rate. As noted above, epigenetic modifiers critically regulate neural crest maturation, and this association with ATRX mutations provides further evidence of disrupted crest developmental pathways driving neuroblastoma.

Non-coding RNAs—Non-coding RNAs (microRNA, lncRNAs, piRNAs) are essential transcriptional regulators of stem cell biology, development and neural crest differentiation. Many of these microRNAs are deregulated in aggressive neuroblastomas and act to block p53 activity and promote EMT and metastasis (36, 68). Recently, neural crest directed

expression of LIN28, an important regulator of microRNA processing, was shown to promote neuroblastoma tumorigenesis in part by inhibiting Let7a microRNA mediated tumor suppression (69). Additionally, miR-9, miR-17-92a, and the miR-25-106b cluster, as well as multiple other microRNAs are directly implicated in either tumorigenesis, metastasis or regulation of differentiation of neuroblastoma and other cancers (70, 71). Numerous studies of the biologic and prognostic impact of microRNAs in neuroblastoma have been reviewed elsewhere (36, 72). Due to the added complexity of non-coding RNAs, defining the entire transcriptomes of neuroblastomas becomes a monumental task; however, it is becoming increasingly possible to integrate coding and non-coding RNA transcriptomic and epigenetic data (e.g., ChIP-seq, and methylome analyses) with similar datasets obtained from models of neural crest development. This should help to further define novel therapeutic targets and pathways common to the heterogeneous subtypes of neuroblastoma.

Future Directions—Tumors are complex collections of varying subtypes of cells, all interacting with each other and the external environment to proliferate and spread. (8). The concepts of CSCs, cellular plasticity and tumor heterogeneity are providing new contexts for investigators to understand the clinical behavior of aggressive cancers such as neuroblastoma (19). Neuroblastoma CSC-like cells recently characterized from murine and human tumor models, as well as primary cancers, demonstrate selective responsiveness to inflammatory cytokines such as granulocyte colony-stimulating factor (G-CSF) and interleukin-6 (IL-6) (73). Signaling by soluble factors and cell-cell interactions between CSCs, non-stem tumor cells, tumor stromal and immune cells must all be considered in order to optimize therapeutic options (21). Disease progression, an all too common an occurrence for neuroblastoma, may be due to inadequately targeting resistant subpopulations. Inflammatory mediators likely promote tumor proliferation and facilitate tissue degradation leading to metastasis. In addition, it is now clear that tumors such as neuroblastoma co-opt the inflammatory immune response to tissue damage, activating and repressing subpopulations of T-cells and macrophages to generate an immune-privileged tumor microenvironment (74). This perspective is guiding the development of novel therapeutics based on adoptive immunotherapy and other approaches to sensitize and redirect the immune system to tumors.

Clinical Integration of Genomic and Biological Data

During the last decade, as neuroblastoma investigators have begun to better understand tumor biology and genetics, they have also begun to use this data to subdivide and risk classify newly diagnosed patients. Working as a collaborative body, the International Neuroblastoma Risk Group (INRG) task force generated a database containing the biological, histological, radiographic and clinical data from 8800 neuroblastoma patients treated across the globe (75). This database has been used to develop the current INRG staging and risk classification schemas that provide an international standard for patient classification. This standard will make it easier for investigators to compare clinical and research data between single institutions, national and international cooperative groups.

Staging and Pre-Treatment Classification

The International Neuroblastoma Staging System (INSS), first published in 1988, used the extent of the initial surgical procedure to define the stage of the patient (76). Small localized tumors that were completely resected were considered Stage I lesions. Stage II tumors were small, may or may not have had lymph node involvement, but could not be completely resected. Stage III lesions were large tumors that crossed the anatomical midline of the patient and could not be completely resected. Lastly, Stage IV and IVS metastatic tumors were differentiated by the fact that IVS patients were <1 year of age with metastatic disease located in liver, skin and less than 10% of the bone marrow (76).

Although used for over 20 years, staging per INSS was influenced by the location of the primary tumor (e.g., intrathoracic vs. abdominal primary), access to experienced pediatric surgery and pathology teams, and access to detailed radiographic imaging. To compensate for these variables, the new INRG Staging System (INRGSS) determines overall staging based on the extent of disease and presence of pre-operative, radiographic, image defined risk factors (IDRFs) that can be used to assess resectability (77, 78). For example, as seen in Table 1, L1 tumors are defined as localized lesions that do not involve vital structures, as defined within the IDRFs, and are confined to 1 body compartment; as compared to L2 tumors that are loco-regional lesions with 1 or more IDRFs.

The current INRG pre-treatment classification schema combines different clinical and biological factors associated with prognosis, including INRG stage, age, histology, tumor differentiation, MYCN status, 11q LOH, and plody to determine a patient's risk group (78). The risk groups have been formally expanded to include 4 categories: very low risk, low risk, intermediate risk, and high risk. The 4 risk groups depicted in Table 2 can then be used to assign treatment recommendations or assess a patient's eligibility for participation on investigational studies (78).

Treatment of Neuroblastoma

Neuroblastoma treatment recommendations range from observation only to intensive, multi-modal therapy and have been based on the event free and overall survival of patients enrolled on large, cooperative group clinical trials. The use of biologic correlates has further allowed the INRG to streamline therapy recommendations. For example, intermediate risk chemotherapy is recommended for patients with L2 tumors, who are <18 months of age, with any histology other than Ganglioneuroma (GN) maturing or Ganglioneuroblastoma (GNB) intermixed histology, and an 11q aberration as compared to a recommendation for observation only for that same patient population without an 11q aberration due to their low risk classification (78). With the impact that biological and genetic information has had on outcome analysis and associated future treatment recommendations, it becomes paramount for investigators to streamline testing on primary tumor and plasma samples while also saving as much material as possible to retrospectively analyze data from scientific discoveries yet to be made.

There have been a number of excellent reviews recently published describing the agents, both chemotherapeutic and immunotherapeutic, used in the treatment of neuroblastoma (79–

81); therefore, the global recommendations for treatment by risk category are described below.

Non-High Risk—With event-free survival outcomes greater than 90% in these patients, there is a concerted effort to identify the subgroups where therapy can further reduced (4, 23, 82–84). In 2012, Nuchtern et al noted that in patients <6 months of age with small, localized adrenal lesions, observation alone was a safe treatment option (22). Of the 84 patients whose lesions were initially observed, 81% spontaneously regressed, and the overall 3-year disease free survival (DFS) was 97.7% (22). This data, coupled with the excellent outcome reported on patients <18 months of age with localized tumors and favorable genomics (hyperdiploid, no chromosome 1p or 11q LOH) treated on A3961 and P9641, is the rationale for the current proposal within the COG to investigate the use of expectant observation in children with a broader age range and slightly larger localized tumor size (4).

The current recommendations for other patients with localized tumors are associated with the presence or absence of IDRFs. Complete excision should be attempted for INRG L1 tumors. Historically larger lesions have been treated with neo-adjuvant chemotherapy in an effort to shrink the tumor prior to surgical resection (77). Low risk lesions with MYCN amplification have a significantly worse EFS (53% vs 72%, P<0.0001) and therefore additional therapy after resection is required (85). The outcomes for patients with local, but unresectable tumors (INRG L2; INSS Stage II or III) are dependent on the histology and biology of the lesion. Results from the COG P9641 and the SIOP European Neuroblastoma Group (SIOPEN) LNESG1 studies both found that patients with INSS Stage II lesions and unfavorable biological features had a worse disease free and overall survival (OS) as compared to those with favorable features (23, 86). In the COG trial, patients with INSS Stage IIB tumors that were either diploid or had an unfavorable histology had a poorer OS as compared to their hyperdiploid or favorable histology counterparts (82). The SIOPEN results noted an EFS and OS difference of 96.4% vs 75.9% and 85.5% vs 61.2%, respectively, for patients with Stage II tumors with favorable vs unfavorable features. Results were similar for those with Stage III disease. Matthay et al showed that older patients with Stage III disease with unfavorable features had a 15% difference in 4-year EFS and 25% in 4-year OS as compared to those with favorable features of any age (65% vs. 80% and 75% vs. 100%, respectively) (87). The COG is considering updating the criteria for high-risk patients to include those over the age of 18 months with INRG L2, MYCN non-amplified lesions, with unfavorable histology and genetics based on this data (4).

A subset of patients with metastatic neuroblastoma is considered non-high risk. Asymptomatic infants with INSS Stage IVS disease can be observed. However, those less than 2 months of age at diagnosis and those with hepatomegaly are at risk for rapid disease progression and strong consideration for the early initiation of chemotherapy should be made in these children (4, 88). The INRG classification schema defines patients <18 months with non-amplified, hyperdiploid stage IV disease as low risk and those of the same age with non-amplified, diploid disease as intermediate risk based on their biological features (78).

High Risk—In general, patients with MYCN amplification and/or those >18 months of age with INRG Stage M disease are considered high-risk. Treatment for patients with high-risk disease is multimodal and involves the use of chemotherapy, surgery, radiotherapy, biologics (cis-retinoic acid) and immunotherapy. Although there (89), the addition of immunotherapy has provided the largest impact on the EF and OS of children who have not progressed prior to that point. In the update by Yu et al to their 2010 report of the Phase III study randomizing patients to cis-retinoic acid alone vs cis-retinoic acid, Interleukin-2 (IL-2), granulocyte-macrophage colony-stimulating factor (GM-CSF) and a chimeric monoclonal antibody targeting GD2 (ch14.18), the use of immunotherapy is associated with an improvement in the 4-year EFS of 48% vs. 59% and OS of 59% vs. 74%, respectively (5, 90). To improve upon these results, other investigators are studying alternative infusion and immunotherapy administration schedules (91, 92).

COG has also considered adding other targeted agents to the next therapeutic protocol for patients with high-risk disease. As previously noted, ALK mutations and aberrations are found in a small percentage of neuroblastoma tumors. Therefore, one potential modification to the treatment schema would be the addition of an oral tyrosine kinase inhibitor targeting ALK for those patients with documented ALK mutations or aberrations.

Many groups are looking for correlative biomarkers to better and more accurately determine treatment responders and non-responders. The 2 most studied indicators include the use of a radiographic, semi-quantitative metaiodobenzylguanidine (MIBG) scoring system and the measurement of minimal residual disease markers in the blood and bone marrow of patients at various time points during treatment (4, 93–95). Once validated, these biomarkers could be important tools to help investigators determine if patients should continue with standard of care treatment plans or be offered alternative treatment options due to their risk of developing relapsed or refractory disease.

Relapsed/Refractory Disease—Treatment of patients with relapsed and refractory disease remains a challenge. Recent data reported by Modak et al shows that patients with a solitary lesion at the time of relapse can be salvaged and recommended using agents with known anti-neuroblastoma activity as compared to experimental therapy in this population (96). However, with the advances in targeted therapies and the understanding that the genetics and biology of tumors at the time of relapse may be different than the original diagnosis, obtaining tumor samples at the time of relapse will be required in order to generate rational treatment choices for children with this disease.

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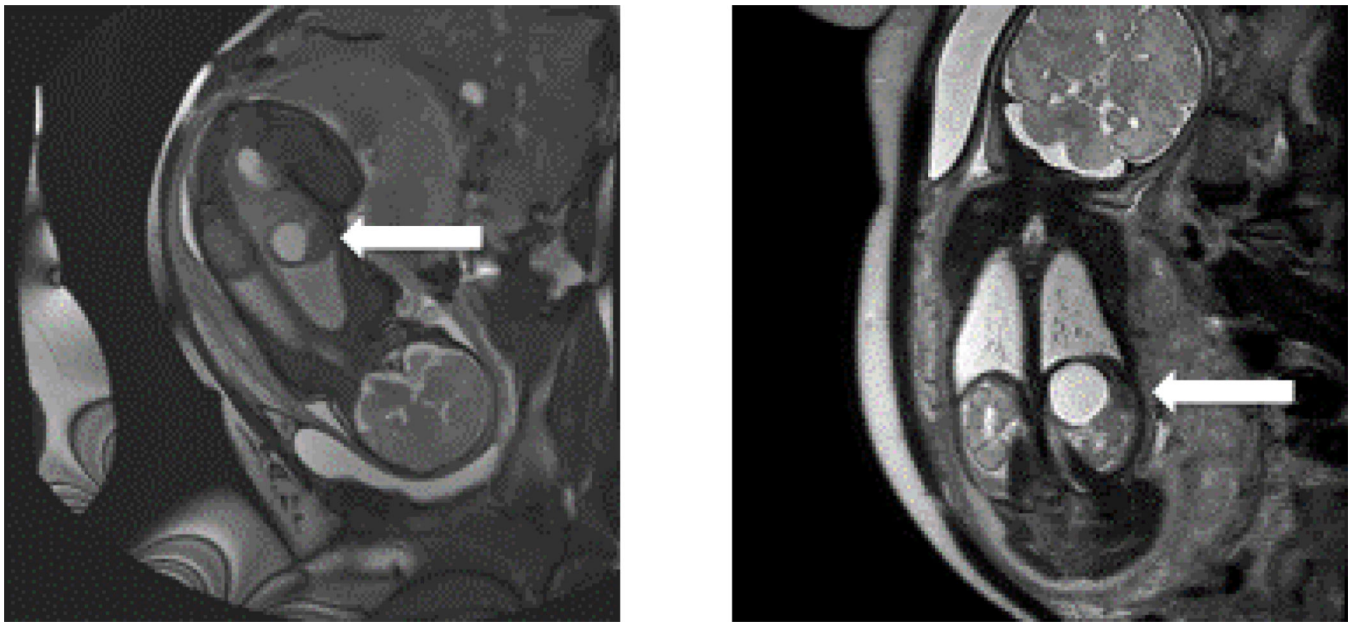

Figure 1.
Cystic adrenal mass identified on a non-contrast fetal MRI obtained at 36 weeks and 2 days of gestation. The white arrows are pointing to a 3.1×3×2.8 cm lesion in the left adrenal gland.

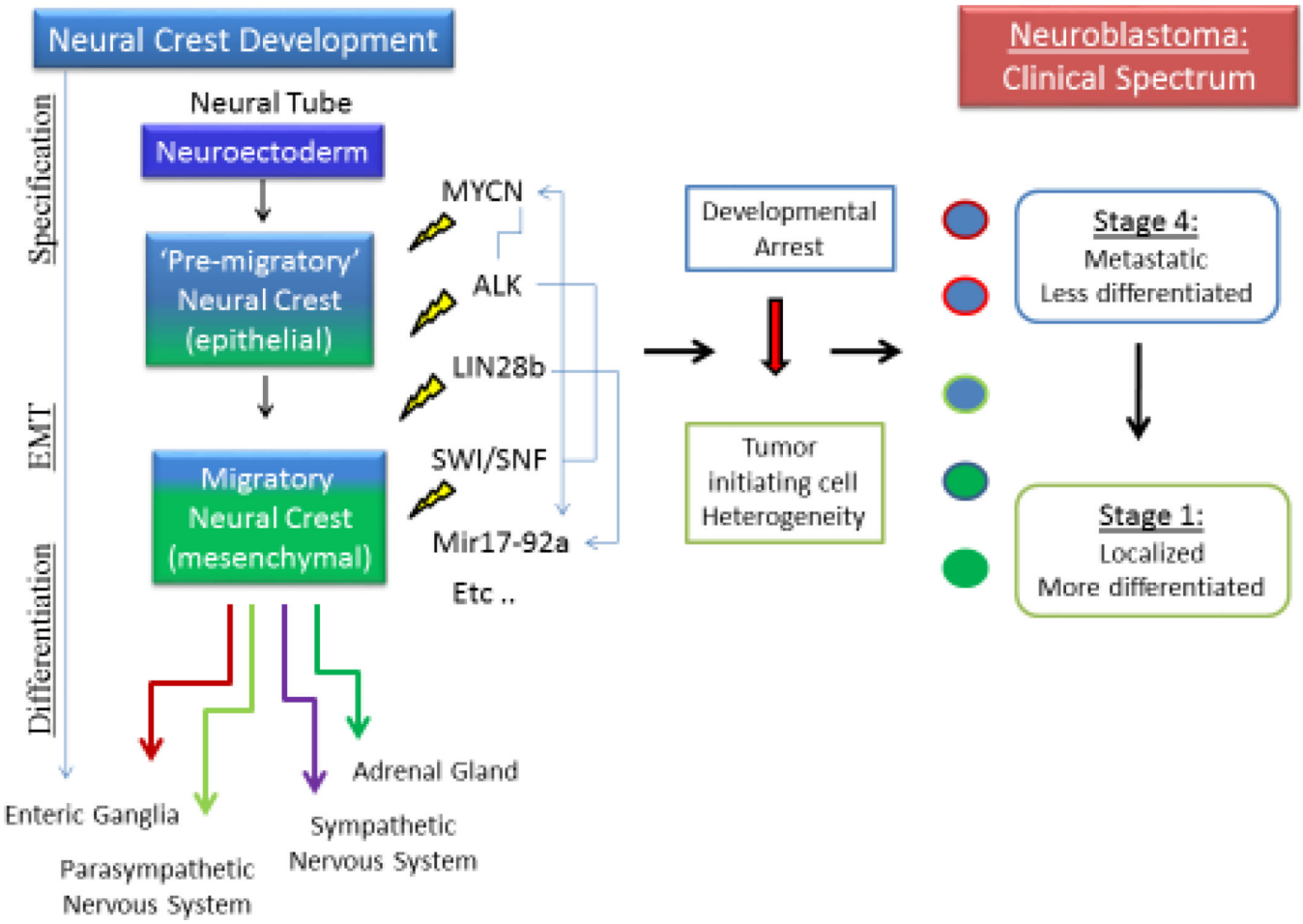

Figure 2.

Neuroblastoma is a spectrum of diseases with a wide range of clinical behaviors. Disruption of the normal maturation progression with different genetic drivers at different times leads to heterogeneity of tumor initiating cells. Interaction between different epigenetic and genetic factors complicates the task of defining a primary oncogenic driver or pathway for this disease. This results in a wide range of pathologies with highly variable responses to treatment.

Table 1

International Neuroblastoma Risk Group (INRG) Staging System from Monclair et al. JCO 2009

Stage	Definition
L1	Localized tumor not involving vital structures as defined by the list of image-defined risk factors and confined to one body compartment.
L2	Locoregional tumor with presence of one or more image-defined risk factors.
M	Distant metastatic disease (except stage MS).
MS	Metastatic disease in children younger than 18 months with metastases confined to skin, liver, and/or bone marrow.

Table 2

International Neuroblastoma Risk Group (INRG) consensus pre-treatment classification schema from Cohn et al. JCO 2009

INRG Stage	Age (months)	Histologic Category	Grade of Tumor, Differentiation	MYCN	11q Aberration	Plody	Pre-treatment Risk Group
L1/L2		GN maturing GNB intermixed					A Very Low
L1		Any, except GN maturing or GNB intermixed		NA Amp			B Very Low K High
L2	<18	Any, except GN maturing or GNB intermixed		NA NA	No Yes		D Low G Intermediate
L2	>18	GN nodular; Neuroblastoma	Differentiating Differentiating Poorly differentiated or undifferentiated	NA NA NA Amp	No Yes		E Low H Intermediate H Intermediate N High
M	<18 <12 12 to <18 <18 ≥18			NA NA NA Amp		Hyperdiploid Diploid Diploid	F Low J Intermediate J Intermediate O High P High
MS	<18			NA NA Amp	No Yes		C Very Low Q High R High

GN: ganglioneuroma; GNB: Ganglioneuroblastoma; NA: Non-amplified; Amp: Amplified; blank field = “any”; diploid (DNA index ≤ 1.0); hyperdiploid (DNA index > 1.0 and includes near-triploid and near-tetraploid tumors); very low risk (5-year EFS > 85%); low risk (5-year EFS > 75% to ≤ 85%); intermediate risk (5-year EFS ≥ 50% to ≤ 75%); high risk (5-year EFS < 50%)