Peripheral neuroblastic tumors (pNTs), which include neuroblastoma, ganglioneuroblastoma, and ganglioneuroma, are common pediatric tumors (Ross et al. 1996). These tumors are derived from immature sympathetic neuroblasts during embryonic, fetal, or early postnatal development, and their morphological features appear to recapitulate developmental stages of sympathetic ganglia. Their primary sites are anatomically related to the embryological distribution of neural crest cells, and include adrenal gland and structures of the sympathetic nervous system.

For many years pNTs were characterized as “enigmatic” because of their unexpected clinical behaviors, such as involution/spontaneous regression, maturation, or aggressive progression. Because of recent advances in clinical and basic research, pNTs now are considered to be biologically heterogeneous, and their individual molecular properties like account for their unique clinical behaviors (Brodeur and Maris 2002). Based primarily on their clinical biology, the International Neuroblastoma Pathology Classification (Shimada et al. 1999a,b) was established by adopting the concept of the original Shimada system (age-linked evaluation of the morphological features in this disease). In this chapter, histopathology of the pNTs is illustrated according to the Classification along with its biological relevance.
8.2 Historical Overview

Since the beginning of the twentieth century, attempts have been made to deduce prognostic information from the histological appearance of the individual tumors (Beckwith and Martin 1968; Hughes et al. 1974; Landau 1911; Mäkinen 1972; Wahl 1914). In 1914 Wahl suggested a sequence of maturation of the pNTs (Wahl 1914), and in 1968, Beckwith and Martin proposed a grading system based on the semi-quantitative assessment of neuroblastic cytodifferentiation (Beckwith and Martin 1968). In 1974 Hughes and co-workers proposed their grading system, but at this time based on non-quantitative assessment of neuroblastic/ganglionic cytodifferentiation (Hughes et al. 1974). As summarized in a review article by Dehner in 1988 (Dehner 1988), however, those attempts could not successfully satisfy the oncologists dealing with this “enigmatic” disease.

Interestingly, in the first half of the twentieth century, it was believed that older patients had a better prognosis (Landau 1911; Wahl 1914), which was probably due to the inability to distinguish local-regional from stage-4 metastatic disease. In the second half of the twentieth century, however, it was clearly recognized that younger patients (especially diagnosed before 1 year of age) had a significantly better prognosis than older patients (Gross et al. 1959). Furthermore, the majority of tumors in infants with clinically favorable outcome showed no or very limited morphological evidence of cytodifferentiation. Beckwith and Martin concluded that “differences in degree of maturation probably did not account for the more favorable outcome of the neuroblastomas in infancy” (Beckwith and Martin 1968).

In 1984 Shimada and colleagues proposed a classification system based on a unique concept of age-linked evaluation of morphological indicators (Shimada et al. 1984). First they made an age-appropriate framework of the maturational sequence of the pNTs. The maturational sequence was defined by two morphological indicators, grade of neuroblastic differentiation, and degree of Schwannian stromal development. Prior to their study, Schwannian stromal component, which is one of the major elements in the normal ganglionic structure of the sympathetic nervous system, had never been a subject of serious investigation in pNTs. According to this classification system, clinically favorable tumors can be less differentiated when diagnosed in younger patients, and should have morphological features of more advanced maturation in older children (for detailed explanation see Chap. 4). They also found increased numbers of karyorrhectic cells in highly aggressive tumors, and introduced a concept of mitosis–karyorrhexis index.

In 1992 Joshi and co-workers proposed histological grading by using mitotic rate (MR: low ≤10/10 high-power fields, high >10/10 high-power fields) and calcification (presence or absence; Joshi et al. 1992). In their report they also proposed a risk grouping by combining the histological grade and age of the patient at diagnosis (low risk: patients in all age groups with tumor having low MR and calcification, and patients ≤1 year of age with either low MR or calcification; high risk: patients >1 year of age with either low MR or calcification, and patients in all age group with high MR and no calcification; Joshi et al. 1992). They later published a modified histological grading by replacement of mitotic rate with MKI (Joshi et al. 1996).

In 1994 the International Neuroblastoma Pathology Committee was formed to establish a prognostically significant and biologically relevant classification for international use. The Committee first defined terminology and morphological criteria of pNTs, and then analyzed and tested mainly those two classifications proposed by Shimada et al. (1984) and Joshi et al. (1992, 1996). In 1999, after 5 years of collaborative work, the Committee developed the International Classification based on the original Shimada classification with minor modifications (Shimada et al. 1999a,b).

8.3 Basic Morphology

As proposed by Shimada et al. in their original classification (Shimada et al. 1984), pNTs are classified into four basic morphological categories (Shimada et al. 1999a): neuroblastoma (Schwannian stroma-poor); ganglioneuroblastoma, intermixed (Schwannian stroma-
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8.3.1 Neuroblastoma (Schwannian Stroma-poor)

Tumors in this category are composed of neuroblastic cells forming lobules separated by thin fibrovascular septa where Schwann cells (or their precursors) can (or may) be detected as slender S-100 positive cells (Shimada et al. 1985). Three subtypes, i.e., undifferentiated, poorly differentiated, and differentiating, are distinguished based on different grades of neuroblastic differentiation. It is noteworthy that in the original Shimada Classification, there were two subtypes, undifferentiated (including undifferentiated and poorly differentiated subtype of the International Classification) and differentiating (same as differentiating subtype), in this category. On gross examination, tumors are generally soft in consistency. Cut surfaces of those in the undifferentiated and poorly differentiated subtype are often hemorrhagic, while tumors in the differentiating subtype are usually tan-yellow, without hemorrhage.

Neuroblastoma, Undifferentiated Subtype (Fig. 8.1a): In this rare subtype, tumor tissue is composed of undifferentiated neuroblastic cells without identifiable neuropil or rosettes. In order to establish the diagnosis, supplementary tests, such as immunohistochemistry, electron microscopy, and/or molecular/cytogenetic analysis, are usually required.

Neuroblastoma, Poorly Differentiated Subtype (Fig. 8.1b): Diagnosis for tumor in this subtype is relatively easy because of the presence of varying amount of neuropil and/or rosettes of the Homer-Wright type. Most of the tumor cells are undifferentiated: less than 5% of the population has morphological evidence of differentiation (see below).

Neuroblastoma, Differentiating Subtype (Fig. 8.1c): Tumor of this subtype usually has abundant neuropil. Five percent or more of the tumor cells are differentiating neuroblasts: they are characterized by synchronous differentiation of the nucleus (enlarged, eccentrically located with vesicular chromatin pattern, and a single prominent nucleolus), and of the cytoplasm (eosinophilic/amphophilic with a diameter two or more times larger than the nucleus).

Mitosis–karyorrhexis index (MKI): One of three MKI classes is assigned to the given neuroblastoma tumor. Those classes are low MKI (<2% or <100 of 5000 mitotic and karyorrhectic cells), intermediate MKI (2–4% or 100–200 of 5000 mitotic and karyorrhectic cells), and high MKI (>4% or >200 of 5000 mitotic and karyorrhectic cells). The MKI is defined by counting the number of tumor cells in mitosis and in the process of karyorrhexis (Fig. 8.1d), and should reflect an average for all tumor sections available. Karyorrhectic cells show condensed and fragmented
Figure 8.1 a–f
Histology of peripheral neuroblastic tumors. 

a Neuroblastoma (Schwannian stroma-poor), undifferentiated subtype.
b Neuroblastoma (Schwannian stroma-poor), poorly differentiated subtype.
c Neuroblastoma (Schwannian stroma-poor), differentiating subtype.
d Neuroblastoma (Schwannian stroma-poor) with a high mitosis–karyorrhexis index.
e Ganglioneuroblastoma, intermixed (Schwannian stroma-rich).
f Ganglioneuroma (Schwannian stroma-dominant), maturing subtype.
nuclear material, usually accompanied by condensed eosinophilic cytoplasm. Simple hyperchromatic nuclei without chromatin fragmentation are not included in MKI counting.

### 8.3.2 Ganglioneuroblastoma, Intermixed (Schwannian Stroma-rich)

The international classification has stipulated that tumors having prominent Schwannian stromal development occupying more than 50% of the tumor tissue are upgraded to this category. Tumor histology is consistent with a transition to the full differentiation/maturation of ganglioneuroma (see below), but the process is not complete, as evidenced by scattered “residual” microscopic foci where neuroblastic cells in various stages of differentiation as well as varying numbers of maturing ganglion cells are found in the background of neuropil (Fig. 8.1e).

### 8.3.3 Ganglioneuroma (Schwannian Stroma-dominant)

Tumors are predominantly composed of Schwannian stroma with individually distributed maturing/mature ganglion cells. Two subtypes, ganglioneuroma, maturing, and mature, are included in this category.

### Ganglioneuroma, Maturing Subtype (Fig. 8.1f)

Tumor of this subtype was previously named “ganglioneuroblastoma, well differentiated” in the original Shimada classification. Some of the neuroblastic components appear to be on their way to fully mature ganglion cells and have appearances of differentiating neuroblasts and/or maturing ganglion cells.

### Ganglioneuroma, Mature Subtype (Fig. 8.1g)

Tumor in this subtype is composed of fully developed Schwannian stroma and mature ganglion cells. Those mature ganglion cells are surrounded by satellite cells. Mature non-myelinating Schwann cells, the dominating component of tumor, characteristically form multiple fascicles covered with perineurial cells.

Tumors categorized as either ganglioneuroblastoma, intermixed, or ganglioneuroma have an elastic consistency, and their cut surfaces are always tan-yellow and homogenous with or without fibrous bands.

### 8.3.4 Ganglioneuroblastoma, Nodular (Composite, Schwannian Stroma-rich/Stroma-dominant and Stroma-poor)

Tumor in this category is characterized by the presence of one or more macroscopic, usually hemorrhagic neuroblastomatous nodule(s) (stroma-poor
component) coexisting with ganglioneuroblastoma, intermixed (stroma-rich component) or with ganglioneuroma (stroma-dominant component; Fig. 8.1h). On microscopic examination, there is typically abrupt demarcation (pushing border or even fibrous pseudo-capsular formation) between the neuroblastomatous nodule(s) and the stroma-rich or stroma-dominant tumor tissue. Some nodules, however, are not clearly demarcated but rather have a zone of neuroblastic infiltration into the adjacent Schwannian stromal tissue. In rare cases the neuroblastomatous nodule becomes so large, dominating the tumor tissue, that one can recognize stroma-rich/stroma-dominant area only by light microscopic examination.

Nodular formation is usually considered to be a feature of the primary tumor, but it may be overlooked on gross examination; thus, those cases with ganglioneuroblastoma, intermixed or ganglioneuroma at the primary site and neuroblastoma at the metastatic site should be classified into this category.

### 8.4 Prognostic Classification

The International Neuroblastoma Pathology Classification (the Shimada system) distinguishes favorable and unfavorable histology groups (Shimada et al. 1999b). Tumors in the favorable histology group fall within a conceptual framework of age-linked maturational sequence from poorly differentiated subtype (<1.5 years of age at diagnosis) to differentiating subtype (<5 years of age) of neuroblastoma (Schwannian stroma-poor) to ganglioneuroblastoma, intermixed (Schwannian stroma-rich) to ganglioneuroma (Schwannian stroma-dominant). The neuroblastoma tumors in this group should have a low (for those patients <5 years of age) or an intermediate (for those <1.5 years of age) MKI. By contrast, tumors in the unfavorable histology group have immature histologies for patient's age and include undifferentiated subtype (at any age), poorly differentiated subtype (≥1.5 years of age), and all subtypes (≥5 years of age) of the neuroblastoma. Among the neuroblastoma tumors, those with a high MKI (at any age) or an intermediate MKI (≥1.5 years of age) also qualify as unfavorable histology. Ganglioneuroblastoma, intermixed and ganglioneuroma are classified into a favorable histology group regardless of the patients' age, although these tumors are usually diagnosed in older children. Ganglioneuroblastoma, nodular (composite, Schwannian stroma-rich/stroma-dominant and stroma-poor) can be divided into two subsets, favorable and unfavorable, by applying the same criteria of age-linked histopathological evaluation to the nodular (neuroblastomatous) component (Peuchmaur et al. 2003; Umehara et al. 2000).

While arriving at the proposed Classification, the International Neuroblastoma Pathology Committee tested other morphological indicators (calcification, mitotic rate) and classifications (original risk grouping by combination of mitotic rate, calcification, and age (Joshi et al. 1992); modified risk grouping by combination of MKI, calcification, and age (Joshi et al. 1996), and analyzed their prognostic effects (Shimada et al. 1999b). Although these indicators and classifications all had prognostic effects by univariate analysis, calcification and mitotic rate did not add any significant prognostic information to the International Neuroblastoma Pathology Classification in multivariate analysis. Furthermore, the Classification could provide significantly better prognostic information than those risk groupings. The Committee also examined the age factor of the Shimada system, and confirmed that the two cutoff points, i.e., 1.5 and 5 years of age at diagnosis, used in the Classification distinguished prognostic groups most significantly (Shimada et al. 1999b).

### 8.5 Biological Relevance

In this section, the biological relevance of International Neuroblastoma Pathology Classification (the Shimada system) is summarized.

#### 8.5.1 Schwannian Development in Neuroblastic Tumors

Peripheral neuroblastic tumors consist of two main cell populations: neuroblastic/ganglionic cells and Schwann cells. As described above, the International Neuroblastoma Pathology Classification uses morphological features of both neuroblastic differentia-
tion and Schwannian stromal development for defining maturational sequence of the pNTs. Based on embryological interactions between normal neuroblasts and Schwann cells (Reynolds and Woolf 1993), some postulate that neoplastic neuroblasts produce Schwann cell mitogens important for their proliferation and development (Ambros 2001). Schwann cells, in return, can secrete anti-proliferative and differentiation-inducing factors crucial to neuronal differentiation. This mutual interaction between neuroblastic cells and Schwannian stromal cells may explain the maturational processes of biologically favorable pNTs. In biologically unfavorable pNTs, there is generally less Schwannian component and limited tumor maturation. The origin of the tumor Schwann cells remains controversial. One study indicates that both cell types, i.e., neuroblastic/ganglionic cells and Schwann cells, arise from the same neoplastic neuroblastic clone or precursor cell (Mora et al. 2001); however, other studies present evidence to support that the Schwann cells in pNTs are reactive in nature and probably recruited from surrounding non-neoplastic tissue by tumor neuroblastic cells (Ambros et al. 1996).

8.5.2 Correlation of Histopathology with MYCN Amplification and trkA Expression

There is a reproducible correlation between the molecular event of MYCN amplification and the morphological manifestations in pNTs (Shimada et al. 1995; Goto et al. 2001). Those tumors with amplified MYCN typically are of the undifferentiated or poorly differentiated subtype of neuroblastoma (Schwannian stroma-poor) with markedly increased mitotic (proliferating) and karyorrhectic (apoptotic) activities (Shimada et al. 1995; Goto et al. 2001), an unfavorable histology group according to the International Neuroblastoma Pathology Classification. The presence of prominent nucleoli in neuroblastic cells of undifferentiated or poorly differentiated neuroblastoma, often associated with unfavorable prognosis (Ambros et al. 2002), can be an additional hallmark of MYCN amplification (own unpublished observations).

The balance appears to favor cellular proliferation (mitosis) more than cellular death (karyorrhexis) in a MYCN amplified tumor, which is well known to have a highly aggressive and rapidly progressive clinical behavior. In light microscopic sections from MYCN amplified tumors, however, the number of karyorrhectic cells always exceeds that of mitotic cells. This may be explained by the fact that the histologically visible stage of mitosis is much shorter than that of karyorrhexis (Bursch et al. 1991). Our preliminary data show that neuroblastoma tumors with favorable histology express significantly higher levels of trkA than those with unfavorable histology (Shimada et al. 2004). Favorable histology neuroblastoma tumors include both poorly differentiated and differentiating subtypes: although there is no difference in the level of trkA expression between these two histological subtypes, tumors of differentiating subtype are diagnosed in significantly older children (usually between 1 and 5 years of age) than those of poorly differentiated subtype (newborn to 1.5 years of age). This may suggest an in vivo latent period required for morphological evidence of neuroblastic differentiation among the neuroblastoma tumors in the favorable-histology group, and supports the concept of an age-linked Pathology Classification.

8.5.3 Composite Tumor

The term “composite” implies that the tumor is composed of histologically and, probably biologically, different clonal populations (Schmidt et al. 1993), a description possibly applicable to almost 10% of pNTs. In the International Neuroblastoma Pathology Classification, this composite form is designated as ganglioneuroblastoma, nodular (composite, Schwannian stroma-rich/stroma-dominant and stroma-poor). In this model the neuroblastic nodule(s) represents the evolution of one or multiple clones, either because of newly acquired genetic aberrations in late stage of tumor progression or the persistence of genetically and biologically distinct variants evolving early in tumor formation. Clinically, two-thirds of these composite tumors are aggressive (Peuchmaur et al. 2003; Umehara et al. 2000).
8.6 Conclusion

Neuroblastic tumors are known to be heterogeneous and their clinical behaviors are driven by complex molecular/genetic properties. The International Neuroblastoma Pathology Classification exploits a system of age-linked evaluation of morphological indicators, to distinguish among tumors with near-identical histological features but vastly different clinical behaviors. This classification offers a unique forum for finding the morphological link between clinical behavior and tumor genetics of the enigmatic cancer of childhood.

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