The Pathology of Adrenal Masses

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Development, Anatomy, and Physiology

Development of the Adrenal Glands

The adrenals are bilateral glands located on the superomedial aspects of the kidney. Two different endocrine tissues types, including the cortex and the medulla, are intimately associated with each other in the adrenal glands. Because of this intimate relationship, hormones from one portion of the adrenal influence other parts of the glands. For example, the synthesis of epinephrine from norepinephrine in the medulla is influenced by the glucocorticoids produced and secreted in the adrenal cortex. The cortex can be detected at 5–6 weeks of gestation in the 9-mm embryo stage. Cortical cells can be detected as proliferations of primitive coelomic cells arising from the peritoneum in the dorsal mesentery [1, 2]. By 8 weeks of gestation the cortical cells appear as a distinct unit with a fibrous capsule separated from the adjacent mesothelium. The fetal cortex is prominent during gestation and forms a distinct fetal zone that is larger than the adjacent definitive cortex. The fetal zone comprises around 75 % of the cortex at birth. The fetal zone involutes during the first 6 months after birth [2]. There are three zones in the adult adrenal gland including the zona glomerulosa, fasciculate, and reticularis. The distinct zones of the cortex are fully developed by the time of puberty. The paraganglionic tissues within the adrenal and in the abdomen arise from the neural crest [3, 4]. The primitive sympathetic cells and nerve fibers invade the paravertebral and paravertebral sympathetic tissue and extend into the adrenal cortex around weeks 5–6 weeks of gestation. The primitive sympathetic cells initially appear as nodular aggregates in the cortex. Chromaffin cells can be identified by 7–8 weeks of gestation. The primitive sympathetic cells peak around 17 and 20 weeks and then the nodules of cells decline subsequently. However, groups of nodular primitive sympathetic cells may persist after birth and into early infancy. The extra-adrenal chromaffin cells involute during the latter part of fetal life and continue to involute after birth [3, 4].

Anatomy and Physiology

Each adult adrenal weighs between 4 and 5 g after the peri-adrenal adipose tissue is carefully removed [5]. Patients with chronic illnesses may have larger glands resulting from prolonged stimulation of the glands secondary to stress [6]. The right adrenal is usually pyramidal in shape

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while the left gland is crescentic or lunate in shape. Cut sections of a freshly prepared adrenal usually reveal a bright yellow outer cortex while the inner cortex is tan. The adrenal cortex in adults constitutes around 90 % of the weight of the gland [7]. The zona fasciculata comprises about 70–80 % of the cortical volume while the glomerulosa makes up about 15 % of the volume. The zona fasciculata and reticularis synthesizes glucocorticoids and sex steroids while the glomerulosa synthesizes mineralocorticoids and is less responsive to adrenocorticotropic hormone (ACTH) than the other two zones. The zona reticularis contains cells with eosinophilic cytoplasm, which can synthesize both glucocorticoids and sex steroids. Ultrastructural examination of the cortex shows cells with abundant smooth endoplasmic reticulum and mitochondria with bulbous cristae, features of steroid-producing cells. The secretion of aldosterone by the zona glomerulosa is regulated by the renin–angiotensin system, while the other two zones are regulated by ACTH. The adrenal medulla in adults occupies about 10 % of the gland volume. Most of the medulla lies within the head of the gland with a smaller portion of medullary tissue within the body. The tail of the adrenal is usually devoid of medullary tissue in the normal gland, but medullary tissue may be present in this region in patients with medullary hyperplasia [8, 9].

Adrenocortical Masses

Adrenal cortical masses may be caused by hyperplasia of the cortex, by primary benign and malignant adrenocortical neoplasms and by other lesions such as cysts of the adrenals, myelolipomas, and metastatic tumors to the adrenal glands.

Adrenocortical Hyperplasia

Hyperplasia Associated with Pituitary ACTH and Hypothalamic CRH Overproduction

An increase in cortical mass may be associated with stimulation of the adrenal cortex by ACTH from the anterior pituitary or from ectopic sources. Excessive production of corticotropin-releasing hormone (CRH) from the hypothalamus or ACTH from the pituitary can lead to hyperplasia of the adrenal cortex primarily in the zona fasciculata and reticularis. ACTH-dependent Cushing syndrome is frequently associated with an ACTH-producing pituitary adenoma (Cushing disease). The left and right glands combined may weigh up to 24 g in markedly severe cases. The hyperplasia is usually diffuse, but a mixed picture of diffuse and nodular hyperplasia may be present. The outer zona glomerulosa is not usually affected by ACTH-dependent hyperplasia. Microscopic examination of the hyperplastic adrenal cortex shows lipid depletion in the zona fasciculata and reticularis. In adults the zona glomerulosa is usually quite compressed and may not be visible, while in pediatric patients this zone may be slightly hyperplastic [10].

Hyperplasia and Paraneoplastic Syndrome

Adrenocortical hyperplasia secondary to paraneoplastic or ectopic hormone production may result from neuroendocrine tumors in multiple sites including the lungs, pancreas, and thymus [11]. Other tumors such as medullary thyroid carcinomas and pheochromocytomas may also be associated with ectopic ACTH and/or CRH production [7]. The adrenals are usually larger on average than in patients with Cushing disease and the combined weight of both adrenals may be up to 30 g. The cortex is diffusely hyperplastic and tan brown. Microscopic examination shows diffuse hyperplasia of the zona fasciculata and the cells appear lipid depleted [7].

Hyperaldosteronism Associated Hyperplasia

Adrenocortical hyperplasia may be associated with hyperaldosteronism. Although adenomas are more commonly associated with hyperaldosteronism, about a third of cases may present with hyperplastic zona glomerulosa cells only or they may be a mixture of adenomas and hyperplasia [12, 13]. The glands may be of variable weight from slight enlargement to a single gland weighing 10 g or more. Microscopic examination

shows proliferation of zona glomerulosa cells and micronodules but zona fasciculata cells may also be present.

Adrenal Macronodular Hyperplasia

This form of hyperplasia is usually bilateral although the glands are often asymmetrically enlarged. The combined weight of the glands may be up to 200 g and the individual nodules may range from less than 1 cm to up to 4 cm in diameter [14–17]. This condition is ACTH independent. Microscopically the nodules may be made up of compact cells, clear cells, or mixture of these [7] (Fig. 2.1a). Interestingly, the cortical tissue between the nodules is atrophic which indicates that the nodules are functional [16]. Massive macronodular adrenocortical disease has been analyzed at the molecular level in a recent study [18]. There were mutations of the armadillo repeat-containing 5 gene (AMRC5) [18].

Pediatric Adrenocortical Disorders Associated with Masses

Adrenal Cytomegaly and Beckwith–Wiedemann Syndrome (BWS)

This syndrome is characterized by hemihypertrophy, macroglossia, abdominal wall defects, and pancreatic islet cell hyperplasia. The adrenal is

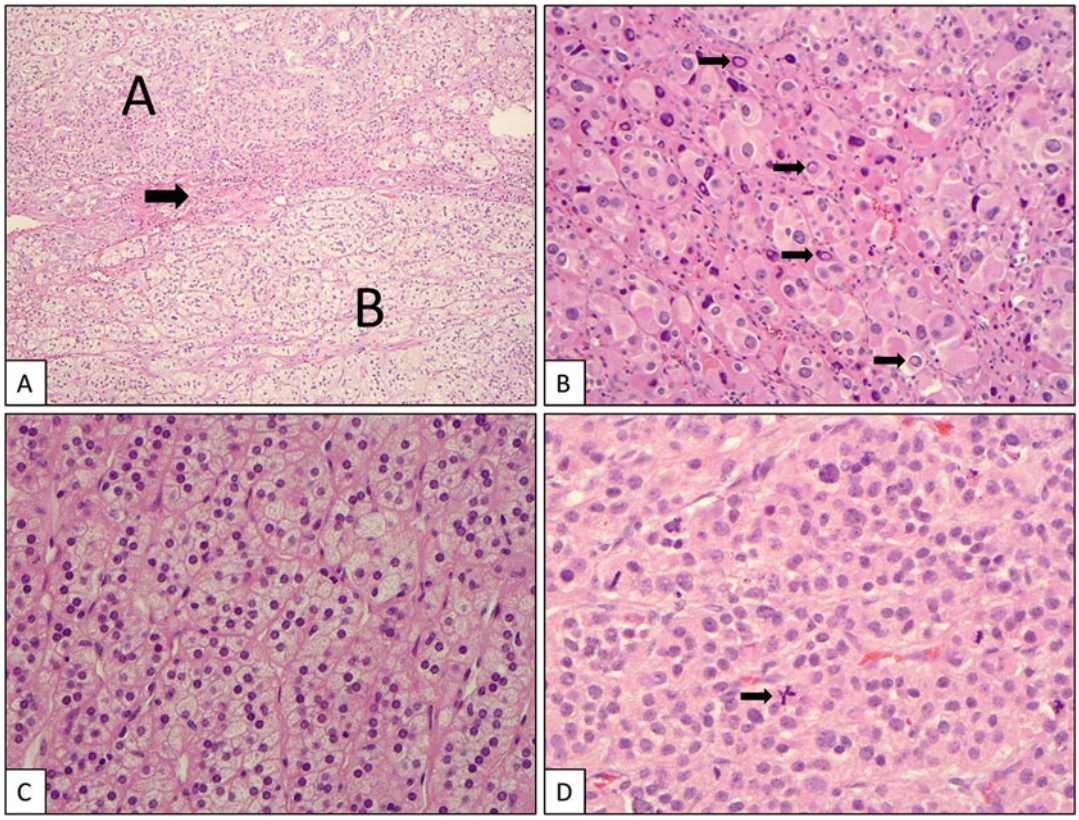

Fig. 2.1 (**a**) Adrenocortical macronodular hyperplasia. Two macronodules are present consisting of fasciculate-like clear cells (letters A and B). (**a, b**) There is atrophy of the adrenal cortex between the two nodules (*arrow*) indicating that the nodules are functional and secreting glucocorticoids. (**b**) Adrenal cortical adenoma from a patient with Beckwith–Wiedemann syndrome. The adrenal cortical cells show marked cytomegaly and there is prominent cytoplasmic invagination into nuclei (*arrows*). (**c**) Histological examination of an adrenocortical adenoma shows cells with abundant clear cytoplasm and round nuclei from the zona fasciculata. (**d**) Adrenocortical carcinoma showing relatively uniform cells with numerous mitotic figures including atypical mitoses (*arrow*)

characterized by collections of enlarged cortical cells with hyperchromatic nuclei in the fetal cortex. Some cells may show intranuclear pseudoinclusions [19, 20]. Although cytomegalic cells are present in the normal fetal cortex, they are more prominent in patients with BWS. These patients have an increased risk of developing adrenocortical adenomas (Fig. 2.1b), carcinomas, neuroblastomas, Wilm's tumors along with hepatoblastomas and pancreatoblastomas. The molecular pathogenesis involves dysregulated gene expression of imprinted genes within the chromosome 11p15 region [21].

Congenital Adrenal Hyperplasia

These disorders result from autosomal recessive enzymatic defects needed in the biosynthesis of adrenocortical steroids. Defects in the activity of various P450 enzymes lead to the syndrome [22, 23]. The most common defect is 21-hydroxylase deficiency (P450 c_{21}). There is inadequate production of glucocorticoids, which leads to adrenocortical stimulation by ACTH secreted from the anterior pituitary. The adrenals are enlarged with a tan-brown color and a cerebriform configuration [7]. Microscopic examination typically shows cortical cells which are lipid depleted. A variant of this disorder, lipoid congenital adrenal hyperplasia, due to mutations of acute regulatory protein, consists of pale yellow adrenals with vacuolated cells and cholesterol clefts and giant cell reaction on microscopic examination [24]. Patients may develop adrenocortical adenomas and carcinomas secondary to congenital hyperplasia [25]. They may also develop testicular tumors [26]. Both adrenal and testicular tumors are dependent on the presence of high serum levels of ACTH and are thus hormone-dependent tumors.

Adrenocortical Neoplasms

Adrenocortical Adenomas

Adrenocortical adenomas are benign neoplasms that may produce hormones associated with any of the hormones produced by the different zones of the adrenal cortex or they may be nonfunctioning. Adrenocortical adenomas are seen at autopsy in a small percentage of cases (around 5 %) [27]. Adenomas may be functional or nonfunctioning. Many of the incidentalomas discovered in patients by imaging studies represent nonfunctioning adenomas. The most common functioning tumors are associated with aldosterone production while adenomas with glucocorticoid production constitute the second most common group [7, 9].

Hyperaldosteronism

Adenomas associated with hyperaldosteronism (Conn Syndrome) are usually small tumors, measuring less than 2 cm in diameter, and they are commonly unilateral. They are often bright yellow on gross examination and may have a thin pseudocapsule separating them from the adjacent cortex. Microscopic examination shows cells with features of zona glomerulosa, fasciculata or reticularis, or a combination of two zones, often referred to as hybrid cells. A characteristic feature seen in many patients treated with spironolactone before surgery is the spironolactone bodies, which are lamellated eosinophilic bodies in the cytoplasm of tumor cells. A distinct halo is often present around these bodies that make them relatively easy to recognize. The tumor cells are usually small with vesicular nuclei and inconspicuous nucleoli, but some adenomas may have cells showing "endocrine atypia" which consist of larger cells and pleomorphic nuclei. Ultrastructural examination usually shows cells with prominent tubular or vesicular mitochondrial cristae and abundant smooth endoplasmic reticulum.

Cushing Syndrome

Benign tumors associated with excess glucocorticoid production and causing Cushing syndrome are usually unilateral discrete masses and can weigh up to 60 g [7, 28]. The size may be quite variable, but they are usually less than 4 cm in diameter. Larger tumors have an increased likelihood of being malignant. On cut section adenomas usually range from yellow to brown without necrosis. Cystic changes may be present especially with

larger tumors. Microscopic examination shows a discrete pseudocapsule with tumor cells arranged in nest and cords. The individual tumor cells resemble cells from the zona fasciculata (Fig. 2.1c). The nuclei are usually round and the cytoplasm contains lipid, giving the appearance of foamy cytoplasmic inclusions. Mitotic figures are uncommon in adenomas. Ultrastructural examination shows cells with abundant smooth endoplasmic reticulum, lipid droplets, and mitochondria with tubulovesicular or vesicular cristae [28]. Clonal analyses have shown that some adenomas are clonal while others may be polyclonal [29].

Adenomas Associated with Adrenogenital Syndromes

Some adenomas may be associated with virilization or feminization. It is important to rule out the possibility of a carcinoma in adrenocortical tumors associated with excessive sex steroid production [9]. Virilizing adenomas usually appear different from adenomas in patients with Cushing syndrome. The tumors are red to brown rather than yellow. Microscopically the tumors have granular eosinophilic cytoplasm without necrosis or significant mitotic figures. Ultrastructural examination shows abundant smooth endoplasmic reticulum and mitochondria with tubular-lamelar cristae.

Nonfunctional Adrenocortical Adenomas

Adrenocortical nodules are not uncommon in surgically resected adrenal glands and can be seen in about 25 % of autopsy specimens [7, 9]. Incidental nodules are also commonly detected with radiographic studies and nonfunctioning nodules are often designated as incidentalomas [30]. Multicentric nodules are often very small nodules, 2–3 cm in diameter and represent the typical nonfunctioning adenomas. They may range from bright yellow to brown with a pseudocapsule. Microscopically they are composed of cells with uniform round nuclei. The cells are reminiscent of fasciculate-type cells of the normal adrenal. On microscopic examination the adjacent cortex does not show atrophic changes as they do with functional adenomas.

Adrenocortical Carcinomas

Adrenocortical carcinomas are uncommon tumors. The incidence is around 1 per million population per year [27]. There is a bimodal distribution of carcinomas: the first occurs in the first two decades of life, and a larger peak is seen in the fifth decade of life [27, 31]. Carcinomas are slightly more common in women than in men [27]. The tumors may be associated with certain familial conditions such as Li–Fraumeni syndrome [32], Beckwith–Wiedemann syndrome [7, 9], and Lynch syndrome [33]. Most adrenocortical carcinomas are functional with estimates as high as 80 % of cases. Adrenocortical carcinomas generally weigh more than 100 g in adults with many tumors weighing more than 750 g [7, 9]. Adrenocortical carcinomas may occasionally be as small as 20–30 g. Carcinomas usually show a nodular appearance and vary in color from bright yellow to pink or brown depending on their lipid content. Areas of necrosis and hemorrhage are not uncommon and some tumors may also show focal areas of calcification. Microscopic examination shows variable patterns of growth ranging from solid to alveolar, and there is often a mixture of various patterns. Microscopic foci of necrosis are common especially in larger tumors. Unusual growth patterns such as a pseudoglandular and spindle shape appearance may be present [7, 9]. The cytoplasm of tumor cells may vary from eosinophilic to vacuolated depending on the lipid content of the cells. Some adrenocortical carcinomas are composed of cells with uniform nuclei, while other tumors may show marked nuclear pleomorphism. Mitotic activity is common including atypical mitoses (Fig. 2.1d). Some carcinomas may show prominent cytoplasmic invagination into the nuclei, but this feature does not have any diagnostic significance.

Immunohistochemical characterization of adrenocortical carcinomas is very important especially in small biopsy specimens [7, 9]. Adrenocortical carcinomas are often positive for keratin especially when very sensitive antigen retrieval techniques are used during the immunostaining procedure. Surprisingly, these tumors are also positive for synaptophysin; a common neuroendocrine

marker, but chromogranin A is consistently negative which helps in the differential diagnosis of pheochromocytomas versus adrenal cortical tumors. Adrenocortical carcinomas are usually positive for inhibin A and for MART1/Melan A. The monoclonal antibody D11 [34] is a good marker for adrenocortical tumors and helps to distinguish them for adrenal medullary tumors. The lymphatic marker D2-40 is usually positive in normal and neoplastic adrenocortical cells while adrenal medullary cells and tumors are negative for D2-40 [7]. The transcription factor steroidogenic factor 1 (SF-1) is another useful marker for adrenal cortical tumors and is relatively specific, since only a few other tissues including some anterior pituitary cells such as follicle stimulating hormone producing cells are also positive for this transcription factor [35]. Ultrastructural examination was historically important in the diagnosis of adrenocortical carcinomas, but with the advent of many relatively specific immunohistochemical stains this approach is no longer used extensively. Ultrastructural features that are helpful in the diagnosis include abundant smooth endoplasmic reticulum characteristic of steroid producing cells and mitochondria with tubular cristae.

Criteria for Malignancy in Adrenocortical Carcinomas

Distinguishing adrenocortical adenomas from carcinomas can be very difficult especially in small biopsy specimens. The studies of Weiss led to the development of histological criteria to separate adenomas from carcinomas of the adrenal cortex [36]. The criteria included necrosis, diffuse architecture, capsular invasion, atypical mitoses, sinusoidal invasion, venous invasion, and mitotic activity per 50 high power fields. Tumors with fewer than two of these criteria never metastasized, while those with more than four almost always recurred or metastasized [36]. Other workers attempted to simplify the Weiss criteria [37] by reducing the number of features needed to make a diagnosis of malignancy. Hough and coworkers had previously developed criteria for the diagnosis of adrenocortical carcinomas [38], but these have not been as robust as the Weiss criteria. Volante and colleagues [39] developed a different set of criteria which included reticulin histochemical staining that was disrupted in carcinomas, but not in adenomas, to separate adrenocortical adenomas from carcinomas. Recent studies have shown that the use of a proliferative index measured by Ki-67/MIB1 can be another useful tool that can assist in separating adenomas from carcinomas [40].

Variants of Adrenocortical Carcinomas

Oncocytic Adrenocortical Carcinomas

Oncocytic adrenocortical carcinoma is a variant of adrenocortical carcinoma that is different enough from usual adrenocortical carcinomas that different criteria for malignancy have been proposed [41]. These tumors are characterized by the presence of abundant cytoplasmic mitochondria. There are major and minor criteria for the diagnosis of oncocytic carcinomas. The major criteria include a high mitotic rate with atypical mitoses and venous invasion. Minor criteria included necrosis, capsular invasion and sinusoidal invasion, and large tumor size. One major criterion was sufficient for the diagnosis of carcinoma, while one to four minor criteria were enough for a diagnosis of tumors of uncertain malignant potential.

Myxoid Variant of Adrenocortical Carcinoma

This variant is characterized by tumors with abundant extracellular myxoid stroma. One study suggested that these tumors have a more aggressive biological behavior compared to conventional adrenocortical carcinoma [42]. In another study, adrenocortical tumors with myxoid stroma usually behaved like carcinomas [43].

Adrenocortical Carcinomas in Pediatric Patients

The criteria used for the diagnosis of adrenocortical carcinomas in adults have not been directly applicable to tumors in the pediatric population. A study from the Armed Forces Institute of

Pathology refined the criteria for diagnosis of pediatric adrenocortical carcinomas [44]. Features that were associated with malignancy in pediatric adrenocortical carcinomas included tumors weighing more than 400 g, tumor size greater than 10.5 cm in diameter, vena cava invasion, capsular and/or vascular invasion, mitotic count greater than 15 per 20 high power fields, presence of atypical mitoses, and confluent necrosis. In multivariate analyses, vena cava invasion, necrosis, and mitotic activity independently predicted malignant behavior [44]. More recently, another group of investigators divided pediatric adrenocortical tumors into low-risk tumors which were confined to the adrenal and weighed less than 200 g, a high-risk group weighing more than 400 g and invading adjacent organs such as kidney and liver and an intermediate risk group weighing between 200 and 400 g confined to the adrenal or weighing less than 400 g with microscopic evidence of invasion into the adjacent soft tissues, and completely resected without evidence of metastatic spread [45].

Molecular Pathology of Adrenocortical Carcinomas

Molecular studies have been used to try to separate adrenocortical adenomas from carcinomas [46–48]. Gene products upregulated in carcinomas include the insulin-like growth factor (IGF) family especially IGF2 and ubiquitin-specific protease 4 (USP4) and ubiquitin degradation1-like (UFD1L). Various genes products are also down-regulated including chemokine ligand 10, retinoic acid receptor responder 2, and aldehyde dehydrogenase family member A1. Some of these gene products may have potential diagnostic importance in separating adenomas from carcinomas. Analysis of microRNAs expressed in adrenocortical adenomas and carcinomas has shown miR483-3p in many but not all carcinomas and this microRNA was overexpressed in only a small percentage of adenomas [49]. Analysis of 37 pediatric adrenocortical carcinomas by whole genome, whole exome, and/or transcriptome sequencing showed that *IGF2* was overexpressed in 100 % of cases and that a dismal outcome was predicted with concomitant *TP53* and *ATRX* mutations [50]. Recent genome-wide analysis of genomic changes in adrenocortical adenomas and carcinomas found more alterations in carcinomas compared to adenomas and identified several novel molecular pathways associated with deregulated genes including oncostatin m. Oncostatin m signaling was identified as a potential target for treatment of patients with disseminated adrenocortical carcinomas [51]. Other molecular studies of adrenocortical carcinomas have reported recurrent alterations in genes not previously reported in adrenocortical carcinomas including *ZNRF3, DAXX, TERT,* and *MED12* [52].

Miscellaneous Adrenal Mass Lesions

Other lesions in the adrenal that should be in the differential diagnosis of mass lesions include adrenal cyst and pseudocysts, adrenal myelolipoma, and metastatic tumors to the adrenal gland [7, 9]. Adrenal cysts and pseudocyst are usually discovered incidentally during CT and MRI studies. Adrenal cysts include epithelial cysts, endothelial or vascular cysts, parasitic cysts, and pseudocysts [53]. Pseudocysts are most common and may vary from a few millimeters up to 10 cm or more in diameter. Pseudocysts do not have a true lining and usually consist of fibrin and hemorrhagic material with a fibrous wall that is sometimes calcified. Adrenal cysts and pseudocysts are almost always nonneoplastic. However, adrenal cortical adenomas, pheochromocytomas, and adrenocortical carcinomas may undergo cystic degeneration, so the wall of cysts should be carefully sampled and examined microscopically to rule out degenerative changes in a cystic neoplasm.

Adrenal myelolipoma is a benign neoplasm of the adrenal gland that is a nonencapsulated mass composed of varying amounts of mature adipose tissue and hematopoietic elements including red and white blood cell precursors and megakaryocytes [54, 55].

Metastatic tumors to the adrenal glands are relatively common and may be present in up to a third of patients with metastatic malignancies [7, 56].

Lung carcinomas are the most common primary site [7, 56]. Other frequent primary sites include breast, skin, kidney, and gastrointestinal tract. Immunohistochemical stains are very useful in determining the primary site of the tumors if the origin is not apparent clinically.

Adrenal Medullary Masses

Adrenal Medullary Hyperplasia

Adrenal medullary hyperplasia may simulate an adrenal mass in some cases [57–59]. Medullary hyperplasia is usually associated with multiple endocrine neoplasia (MEN)2A and MEN2B [57–59]. Medullary hyperplasia in von Hippel Lindau (VHL) disease and neurofibromatosis, both of which are associated with bilateral pheochromocytomas, is somewhat controversial [60, 61]. Occasional patients may have medullary hyperplasia without a family history. The hyperplasia in MEN2A and MEN 2B is usually diffuse and nodular. Morphometric studies may be needed to document a diagnosis of medullary hyperplasia in mild cases. Medullary hyperplasia may be suspected when medullary tissue is present in both alar regions of the adrenal and medullary tissue is also present in the tail of the adrenal [7]. Microscopically the medullary cells are composed of enlarged polygonal cells with abundant granular cytoplasm and round nuclei. Immunostaining with chromogranin A may assist in outlining the extent of the medullary tissue extension in the adrenal glands. Recent molecular studies have shown that in patients with MEN2A the nodular hyperplasia is clonal, suggesting that the nodules represent true neoplasm rather than simply hyperplastic nodules [7].

Pheochromocytomas

Pheochromocytomas are also referred to as intra-adrenal paragangliomas. Most pheochromocytomas arise in the adrenal glands. Tumors with similar histological features outside of the adrenal glands have been designated as pheochromocytomas by some investigators, but they should be termed as paragangliomas or extra-adrenal paragangliomas. Pheochromocytomas vary in color from gray to pink. After exposure of the cut section to air, the surface may appear brown due to oxidation of catecholamines or adrenochromes in the tumor. Tumors may vary from around 1 to 5 cm or more. Malignant tumors are generally larger than benign pheochromocytomas. Areas of degeneration and fibrosis may be present in larger pheochromocytomas; however, these degenerative foci should not be misinterpreted as a sign of malignancy. Almost all sporadic pheochromocytomas are unilateral while familial tumors are generally bilateral and some of these are associated with medullary hyperplasia. Microscopically the tumors consist of large polygonal cells with granular cytoplasm (Fig. 2.2a). The tumors are usually basophilic, but the color varies with the fixation. Pheochromocytomas may rarely show oncocytic or clear cell features. The clear cell variant may be confused with adrenal cortical neoplasm, so immunohistochemical stains should be performed especially with small biopsies.

Other histological variants may include small cell and spindle cell variant of pheochromocytoma. A distinct feature of pheochromocytomas is the presence of eosinophilic globules that may be in the cytoplasm or in between adjacent tumor cells. The globules are thought to be derived from the membrane of secretory granules [7, 9]. These globules are not unique to pheochromocytomas, since they can also be seen in normal adrenal medulla and even in adrenocortical carcinomas. The sustentacular cells located at the periphery of nests of pheochromocytomas when the pheochromocytes form a zellballen pattern or cell nest is another component of pheochromocytomas. The function of the sustentacular cell is unknown, but they have been reported to be decreased in malignant pheochromocytomas. Sustentacular cells can be readily recognized by their spindle shape at the periphery of the cell nests or by immunostaining for S100 protein. The nuclei of pheochromocytoma cells are round to ovoid and they may show cytoplasmic invagination from the cytoplasm into the nucleus in some cells. Amyloid has been reported in varying percentages of pheochromocytomas and in some series may be as high as in 70 % of cases [62].

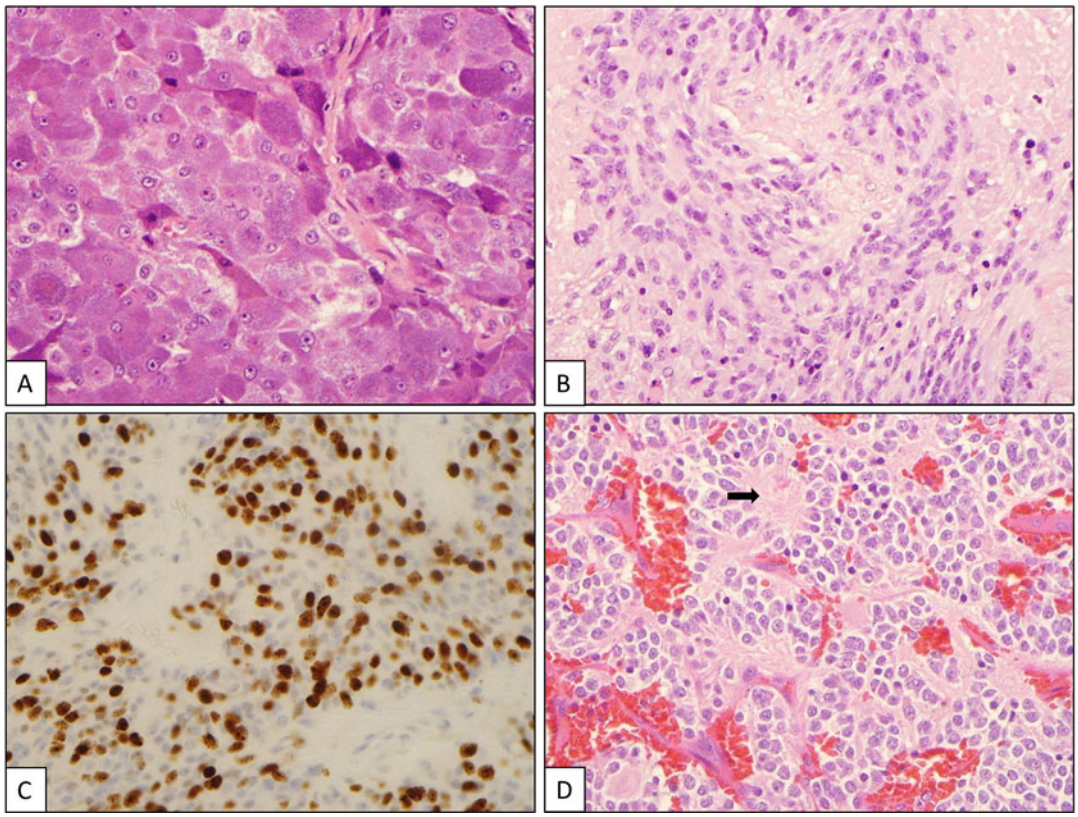

Fig. 2.2 (**a**) Pheochromocytoma with large polygonal cells with granular basophilic cytoplasm and round nuclei. (**b**) Malignant pheochromocytoma with proven liver metastasis. The tumor cells are predominantly spindle shaped and there is extensive background necrosis. (**c**) Immunostaining for Ki-67 in the same pheochromocytoma shows a high proliferative rate (42%) as indicated by brown nuclear staining. (**d**) Neuroblastoma, poorly differentiated, with prominent neuropil and Homer Wright pseudorosettes (*arrow*). Many congested capillaries with red blood cells are present in the background

Immunohistochemical staining is usually helpful in the diagnosis of pheochromocytomas. The tumors are usually positive for chromogranin A, Chromogranin B, and secretogranins. Although pheochromocytomas are also positive for synaptophysin, this marker is less specific since adrenal cortical tumors are often positive for synaptophysin. Antibodies directed against catecholamine synthesizing enzymes such as tyrosine hydroxylase, dopamine beta hydroxylase, and phenylethanolamine, and N-methyltransferase may help in the diagnosis of pheochromocytoma [7]. Pheochromocytomas are usually negative for keratins, although focal positivity may be present. Vimentin and neurofilament are usually positive in pheochromocytomas as is OCT3/4, which is a marker for some germ cell tumors and stem cells [63]. GATA 3 is variably expressed in pheochromocytomas [64]. Regulatory peptides may be detected in pheochromocytomas and may lead to paraneoplastic syndrome. The more common peptides include ACTH, CRH, somatostatin, and calcitonin [7, 9]. Ultrastructural studies of pheochromocytomas show the presence of dense core secretory granules ranging in size from 200 to 800 nm in diameter. Norepinephrine- and epinephrine-containing secretory granules can be distinguished by their morphological appearance. Norepinephrine-containing secretory granules usually have a halo around the secretory granule contents of the membrane [7]. The smooth endoplasmic

reticulum is less developed in pheochromocytomas compared to steroid-producing adrenocortical tumors.

Composite Pheochromocytoma

Composite pheochromocytomas are rare variants of pheochromocytomas. They usually consist of pheochromocytomas and ganglioneuromatous elements but may also be composed of neuroblastic, ganglioneuroblastic, or malignant nerve sheath elements in addition to pheochromocytes. The pheochromocytes usually comprise the majority of the neoplasm. Composite pheochromocytomas with ganglioneuromas are usually benign neoplasms [7, 9]. They consist of pheochromocytes with neuronal or ganglion cells features along with a loose fibrillary matrix resembling neurophil. Transition between the different elements may be gradual or abrupt. The ganglionic cells may contain granular basophilic material corresponding to Nissl substance. The ganglionic cells are characterized by light pink eosinophilic cytoplasm with distinct borders and rounded eccentric nuclei and prominent nucleoli. Prominent Schwann cells are often present. Many of the reported composite pheochromocytomas have been functionally active with secretion of catecholamines. They may be associated with vasoactive intestinal polypeptide secretion leading to watery diarrhea [7, 9].

Paragangliomas

Paragangliomas are generally classified as parasympathetic or sympathetic tumors. The parasympathetic paragangliomas include tumors from the carotid body, jugulotympanic, vagal, laryngeal, aortopulmonary, and miscellaneous tumors in the head and neck region [7, 9, 65]. The tumors are usually firm and solid with a compressed pseudocapsule. On cross section they have a light brown to tan appearance and may have intersecting bands of fibrous tissues. Microscopically it is not possible to determine the anatomic location of different tumors from their histological appearance. An organoid appearance is typical and the cytoplasm is often eosinophilic. Nuclear pleomorphism and hyperchromasia may be prominent and are not reliable criteria for evaluating malignancy. Necrosis is usually not a consistent feature in most paragangliomas. If abundant necrosis is present, one should consider the likely possibility that the tumors were embolized before surgery. The sustentacular cells are similar to those in pheochromocytomas and are positive for S100 protein. The chief cells are positive for chromogranin and synaptophysin.

Sympathoadrenal paragangliomas arise predominantly in the retroperitoneum from the upper abdomen to the pelvic floor. The anatomic region corresponding to the organs of Zuckerkandl at the bifurcation of the aorta has the highest volume of paraganglionic tissue outside of the adrenal medulla. Other anatomic sites of these paragangliomas include the urinary bladder, gall bladder, spermatic cord, prostate glands, pancreas, uterus, and renal hilum. The gross appearance of the tumors is well circumscribed and may appear encapsulated [9]. They may range from a few centimeters in diameter such as in the urinary bladder to 8 cm or larger. Functional tumors are generally smaller than the nonfunctional ones. Microscopically the tumors consist of anastomosing cords of cells or trabecular arrangement with acidophilic granular cytoplasm. Nuclear pleomorphism may be prominent. Nuclear pseudoinclusions are more common than in head and neck paragangliomas. Ganglion-like cells may be present in some tumors. Rarely, paragangliomas and pheochromocytomas may have a black appearance due to the presence of melanosomes and premelanosomes on ultrastructural examination.

Multicentric and Familial Paragangliomas

Familial paraganglioma syndromes are associated with the succinate dehydrogenase gene family mutations including *PGL1 (SDHD), PGL2 (SDHAF2), SDHC*, and *PGL4 (SDHB). PGL* 1, 2, and 3 are associated with paragangliomas of the head and neck region. *PGL1* is the most common paraganglioma syndrome and is associated with a

low incidence of malignancy while *PGL4* with mutations of *SDHB* has the greatest association with malignancy [66].

Malignant Pheochromocytoma/ Paragangliomas

Diagnosis of malignancy in pheochromocytomas/paragangliomas is very difficult, since there are no absolute criteria to predict the behavior of these neoplasms. Earlier studies suggested that features more commonly associated with malignancy included larger tumor size with a mean weight of 383 g for malignant tumors compared to 73 g for benign tumors, vascular invasion, confluent tumor necrosis, and extensive local invasion [7, 9, 67]. Other features that were less specific in distinguishing benign from malignant tumors included decrease number of hyaline globules in malignant pheochromocytomas and decrease numbers of sustentacular cells in malignant tumors [7, 9]. However, there was usually a great deal of overlap of these features in benign and malignant pheochromocytomas/paragangliomas.

Other approaches have been used to try to separate benign and malignant pheochromocytomas/paragangliomas. The use of cytomorphometry was attempted for some time, since benign tumors had a mode corresponding to diploid population of DNA content and a wide range of values with nuclei up to 40n. In contrast, malignant pheochromocytomas were hyperdiploid or triploid with a smaller range of values [7]. Cytomorphometric analysis is not currently widely used in separating benign and malignant pheochromocytomas. The proliferating marker Ki-67/MIB-1 has been used as an adjuvant marker in separating benign and malignant pheochromocytomas (Fig. 2.2b, c). Because the proliferation rate of pheochromocytomas is relatively low, a cut point of 3 % has been used in separating the two groups [7]. A recent study has used Ki-67 as one of several parameters in separating benign and malignant neoplasm as will be discussed later.

Pheochromocytoma of the Adrenal Gland Scaled Score (PASS)

Thompson [68] proposed the pheochromocytoma of the adrenal gland scaled score (PASS) system to separate benign from malignant pheochromocytomas based on a clinicopathologic and immunophenotypic study of 100 cases. Fifty histologically malignant and 50 histologically benign pheochromocytomas of the adrenal gland were studied. Histologically, the cases of malignant pheochromocytomas of the adrenal gland demonstrated larger size, invasion such as vascular, capsular, and periadrenal adipose tissue spread, large nests or diffuse growth, and focal or confluent necrosis, high cellularity, tumor cell spindling, cellular monotony, increased mitotic figures, atypical mitotic figures, profound nuclear pleomorphism, and hyperchromasia more frequently than benign tumors. The PASS system weighted for these specific histologic features could be used to separate tumors with a potential for a biologically aggressive behavior (PASS greater or equal to 4) from tumors that behave in a benign fashion (PASS <4). The pathologic features that are incorporated into the PASS correctly identified tumors with a more aggressive biologic behavior [68].

PASS was one of the earlier scoring systems for the diagnosis of adrenal pheochromocytomas. However, the reproducibility and clinical significance of the PASS system has been controversial [69, 70]. One study [69] found that a higher threshold of 6 was indicative of malignant behavior but recommended that patients with a PASS score 4 should be closely followed. Another group of pathologists [70] examined the utility of PASS by reviewing an independent single institutional cohort of adrenal pheochromocytomas as evaluated by 5 multi-institutional pathologists with at least 10-year experience in endocrine pathology. Significant interobserver and intraobserver variability in the PASS score with variable interpretation of the underlying components was reported, suggesting that this was not a very reliable approach even for expert endocrine pathologist [70].

Grading System for Adrenal Pheochromocytomas and Paragangliomas (GAPP System)

The Pheochromocytoma Study Group in Japan analyzed 163 tumors including 40 metastatic pheochromocytomas and paragangliomas using their grading system for adrenal pheochromocytoma and paraganglioma (GAPP) System [71]. The tumors were scored based on GAPP criteria as follows: histologic pattern, cellularity, comedotype necrosis, capsular/vascular invasion, Ki67 labeling index, and catecholamine type. All tumors were scored from 0 to 10 points and were graded as one of three types: well differentiated, moderately differentiated, and poorly differentiated. GAPP scores of the nonmetastatic and metastatic groups were 2.08 ± 0.17 and 5.33 ± 0.43, (mean ± SE, $P < 0.001$), respectively. The mean number of years until metastasis after the initial operation was 5.5 ± 2.6 years. The 5-year survival of these groups was 100, 66.8, and 22.4 %, respectively. In addition, negative immunoreactivity for succinate dehydrogenase gene subunit B (SDHB) was observed in 13 (8 %) moderately or poorly differentiated tumors, and 10 of the 13 (77 %) had metastases [71].

Molecular Alterations in Pheochromocytomas/ Paragangliomas

Familial pheochromocytomas are associated with MEN2A, MEN2B, VHL disease, and neurofibromatosis type I (NF1) [7, 9, 71]. In MEN2A and 2B disease there is a germline activating mutation in the *RET* protooncogene. In VHL-associated pheochromocytomas, the *VHL* gene usually contains a mutation at codon 238 [72]. Patients with NF-associated pheochromocytomas usually have mutation of the *NF1* gene [73]. Recent studies of pheochromocytomas/paragangliomas have reported germline or somatic mutations in *THEM127, H-RAS, KIF1B, HIF2* alpha, *PHD2*, and fumarate hydratase *(FH)* genes in addition to the *RET, VHL,* and *NF1* genes [66].

Mutations in the succinate dehydrogenase (SDH) mitochondrial complex II, an enzyme complex that catalyzes the oxidation of succinate to fumarate in the Krebs cycle and participates in the electron transport chain, are present is some pheochromocytomas as well as in paragangliomas. *SDHx* genes are composed of four subunits encoded by the corresponding genes: *SDHA, SDHB, SDHC*, and *SDHD*. Complex subunits A and B constitute the catalytic core of the enzyme, while subunits C and D anchor the complex to the inner mitochondrial membrane. In general, inactivating mutations in one of the *SDHx* genes lead to accumulation of succinate and formation of reactive oxygen species, stabilizing HIF1 protein and activating hypoxia-dependent pathways [74, 75]. The few individuals with *SDHA* mutations described so far have presented with distinct phenotypic characteristics of pheochromocytomas/paragangliomas including sympathetic (abdominal and thoracic], and parasympathetic head and neck paragangliomas. Both missense and nonsense mutations have been reported without any genotype–phenotype correlations [74–78].

SDHB mutations have been reported in some intra-adrenal tumors, but mostly in extra-adrenal sites. Recurrence and malignancy were strongly associated with *SDHB* mutations and suggested that the presence of *SDHB* mutants should be considered a high-risk factor for malignancy or recurrence [77–80]. *SDHD* mutation is associated with head and neck paragangliomas. As with other familial paragangliomas, these patients are more likely to have multifocal disease. Both *SDHB* and *SDHD* mutated gene-related pheochromocytomas and paragangliomas typically secrete norepinephrine and dopamine, or dopamine alone. *SDHC* mutations were initially described in head and neck paragangliomas, but have since been reported in adrenal pheochromocytomas and paragangliomas at other sites [80, 81].

Several investigators have reported the use of immunohistochemical methods with antibodies to the SDHx proteins to screen for mutations of *SDHB, SDHC,* and *SDHD* genes in familial disease associated with the pheochromocytoma-paraganglioma syndrome [82, 83]. Loss of SDHx expression is suggestive of a mutation with these antibodies. An internal control such as endothelial

cells should show positive staining for SDHx to support the immunohistochemical method. These screening methods should be validated with more conventional molecular screening to detect the specific mutation.

MicroRNA profiling has been used to try to separate benign and malignant pheochromocytomas [84, 85]. MiR-483-5p was reported to be overexpressed in malignant tumors while miR-15a and miR-16 were underexpressed in the malignant tumors [84]. Another study also observed overexpression of miR-483-5p as well as miR-183 and miR-101 in malignant pheochromocytomas compared to benign tumors [85]. A comprehensive genomic landscape analysis of pheochromocytoma/paraganglioma indicated that the main drivers were distinct germline and/or somatic mutations in susceptibility genes and unique gene alterations were noted [86]. There were miRNA clusters 182/196/183 which were associated with *SDHB*-mutated tumors and were associated with some malignant traits while silencing of the imprinted DLK1-MEG3 miRNA cluster, which included a long noncoding RNA, was noted in a specific subgroup of sporadic tumors [86].

Peripheral Neuroblastic Tumors

Peripheral neuroblastic tumors (pNTs) refer to a group of tumors including neuroblastoma, ganglioneuroblastoma, and ganglioneuroma, with neuroblastoma accounting for 97 % of the cases. They are by definition embryonal tumors of the sympathetic nervous system arising from the neural crest [87]. They almost exclusively occur in children and are the most common solid extracranial tumor in children [88]. Due to its childhood onset, multiple maternal and prenatal factors have been investigated and suggested to be pathogenic, including prenatal exposures to tobacco, alcohol and pesticides, maternal medication or drug use, folate deficiency, gestational diabetes mellitus, small size for gestational age, congenital abnormalities, and maternal history of fetal loss. However, none of these possible associations were confirmed in large studies [87]. pNTs typically arise in the adrenal medulla, paravertebral sympathetic ganglia, and sympathetic paraganglia, with the adrenal being the most common site and accounts for 40 % of the cases [88]. They are heterogeneous tumors and demonstrate many unique behaviors, including involution/spontaneous regression and maturation. Due to their prevalence in children and the aggressiveness of some cases, nation-wide screening programs based on biochemical profiling were lunched in different regions. However, so far only the screening programs in Japan focusing on older children showed some potential benefits [81, 88]. Early detection of pNTs is particularly challenging. The once attractive concept of in situ neuroblastoma, small nodules of neuroblastic cells found within the adrenal gland of asymptomatic children, were found to be in fact remnant of normal fetal development [89]. Recent studies revealed that malignant neuroblastomas and developing neuroblasts share similar genetic profiles [90], reinforcing the difficulty of early diagnosis of pNTs at the molecular level.

Classification

International Neuroblastoma Pathology Classification divides pNTs into four categories based on the level of differentiation and the arrangement of the undifferentiated components: neuroblastoma (Schwannian stroma-poor); ganglioneuroblastoma, intermixed (Schwannian stroma-rich); ganglioneuroma (Schwannian stroma-dominant); and ganglioneuroblastoma, nodular (composite Schwannian stroma-rich/stroma-dominant and stroma-poor) [91]. Grossly, pNTs demonstrate considerable variations but are usually solid, white tan, with varying amounts of hemorrhage, necrosis, cystic degeneration, and calcification. Typically, the less mature component of pNTs is grossly associated with increased hemorrhage and/or necrosis and vague borders. Neuroblastomas are the least differentiated class of pNTs and are usually hemorrhagic with vague, bulging lobules. Ganglioneuroblastomas are more homogenous than neuroblastomas, and more frequently demonstrate calcification and cystic

degeneration. Different from the intermixed type, the nodular type of ganglioneuroblastomas by definition has grossly identifiable hemorrhagic nodules with well-demarcated borders, corresponding to less differentiated area. Ganglioneuromas are the most differentiated tumors among the pNT spectrum, usually well circumscribed with resilient texture, trabecular or whorled appearance, and minimal hemorrhage.

Microscopically, the classification of pNTs reflects the presence and amount of Schwannian stroma as well as the cytological features of the tumor cells. Neuroblastomas contain none or minimum of up to 50 % of Schwannian stroma. They are further divided into undifferentiated, poorly differentiated, and differentiating subtypes. Undifferentiated subtype of neuroblastomas is filled with small to medium size blue monotonous cells with minimal cytoplasm on H&E staining. The nuclei have the characteristic salt-and-pepper appearance and may contain distinct nucleoli. Identifiable background thin neuritic process (neuropil) is absent. Tumors of the poorly differentiated subtype contain mainly undifferentiated cells (Fig. 2.2d). They are separated from the undifferentiated subtype by the presence of neuropil and less than 5 % of differentiating tumor cells that have appreciable nuclear enlargement, eosinophilic cytoplasm, and clearer cell borders. Tumors of the differentiating subtype are further matured compared to poorly differentiated subtype, containing more than 5 % of differentiating tumor cells. Ganglioneuroblastomas by definition contain more than 50 % of Schwannian stroma. The tumor population consists of a mixture of more than 5 % of the differentiating and undifferentiated tumor cells, with the latter forming either microscopically (intermixed) or macroscopically (nodular) distinct clusters. Ganglioneuromas do not contain any undifferentiated tumor cells and are with dominant Schwannian stroma. They are further divided into maturing (when still contain differentiating neuroblasts) and mature (when no longer contain any neuroblasts) subtypes [91–95].

pNTs are usually considered "enigmatic" because the standard grading and staging systems often fail to predict clinical behaviors, especially the occurrence of involution. It was gradually realized that in addition to the level of differentiation, patient's age, cellular turnover index (reflexed by mitosis-karyorrhexis index, MKI, defined by the number of cells with mitosis and karyorrhexis of every 5000 cells), and the presence of macroscopic nodules of neuroblasts are important factors for risk stratification. This knowledge is summarized in International Neuroblastoma Pathology Classification (Table 2.1), which was proposed by International Neuroblastoma Pathology Committee based on the Shimada classification [91] in 2001 [93, 94] and was subsequently revised in 2003 [95]. Based on histologic features and the age at diagnosis, pNTs can be divided into those with "favorable histology" (FH) and "unfavorable histology" (UH). In general, increased tumor cell differentiation, younger age (<18-month old), and low MKI (<2 %) are associated with FH; while decreased tumor cell differentiation, older age (>5-year old), and high MKI (>4 %) are associated with UH. It was originally noted that the presence of macroscopic nodules of neuroblasts within ganglioneuroblastoma conveys a universal unfavorable prognosis [91]. However, subsequent analysis revealed that the observation was not entirely accurate and younger patients with macroscopic nodules of poorly differentiated or differentiating rather than undifferentiated neuroblasts may still have favorable prognosis [95].

Table 2.1 Prognosis of poorly differentiated and differentiating neuroblastoma [95]

Subtype	MKI	Age (year)	Prognosis
Poorly differentiated	>4 %	Any	UH
	Any	>1.5	UH
	<4 %	<1.5	FH
Differentiating	Any	>5	UH
	<4 %	<1.5	FH
	>4 %	Any	UH
	<2 %	1.5–5	FH
	>2 %	1.5–5	UH

MKI mitosis-karyorrhexis index, *UH* unfavorable histology, *FH* favorable histology

Molecular Pathology of pNTs

A familial history of pNTs is observed in about 1% of patients with pNTs and autosomal-dominant inheritance is suggested. Three genes involved are PHOX2B, ALK, and NF1, which are also important for normal development and differentiation of neural crest. Hirschsprung's disease, Congenital Central Hypoventilation Syndrome, Noonan syndrome, and Costello syndrome are known to be associated with increased risk of pNTs [96]. Single nucleotide polymorphisms (SNPs) of several additional genes were suggested to be related to the susceptibility of pNTs, including *BARD1, LINC00340, LMO1, DUSP12*, *DDX4/IL31RA, HSD17B12, LIN28B, HACE1, CHEK2, PINK1,* and *BARD1*. However, the risk that neuroblastoma may recur in families with these risk alleles is estimated to be very low [87]. The presence of recurrent somatic mutations in sporadic pNTs is interestingly low, and very few genes were suggested to separate low- and high-risk pNTs. In the most recent effort to characterize recurrent mutations in sporadic pNTs by whole genome sequencing and exome sequencing, it was found that the median exonic mutation frequency was only 0.60 per Mb, and somatic mutations in only a few genes, namely, *ALK, PTPN11, ATRX*, *MYCN,* and *NRAS*, are associated with high-risk pNTs at low frequency [97]. It is possible that the majority of high-risk pNTs are driven by rare germline variants, copy number alternations, and epigenetic modifications.

MYCN amplification is seen in about 20–30% cases of pNTs and was known for its association with unfavorable prognosis [91, 93]. It is frequently seen in pNTs with advanced stage and rapid progression. The ploidy of tumor cells also appears to affect prognosis, with hyperdiploidy being associated with favorable prognosis. Both *MYCN* amplification and ploidy are included in the current Children's Oncology Group Risk Group Classification [87, 98]. Possible prognostic chromosomal abnormalities include loss of heterozygosity of chromosome 11q, 1p, 14q and gain of 17q [87, 99, 100]. In terms of additional prognostic genetic permutations, *TRKB (NTRK2)* transcript is suggested to be expressed primarily in highly aggressive *MYCN*-amplified tumors [87, 90]. Due to the routine need of analysis of *MYCN* amplification and ploidy in pNTs with snap-frozen tissues and cell culture in addition to histological examination, it is advisable to provide sufficient tissues when obtaining a biopsy [89].

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