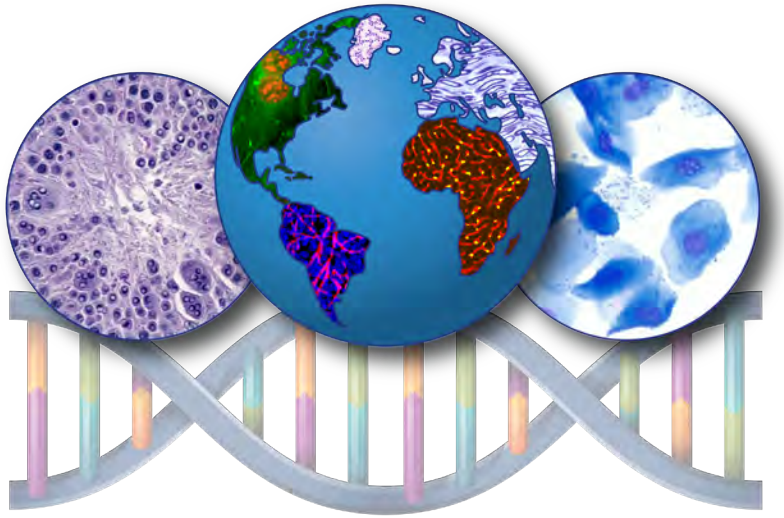
National Institute of Environmental Health Sciences
Division of Translational Toxicology

Proliferative Lesions in the Rodent Adrenal Gland



Division of Translational Toxicology Global Toxicologic Pathology Training Program

National Institutes of Health • U.S. Department of Health and Human Services

Proliferative Lesions of the Adrenal Gland - Overview

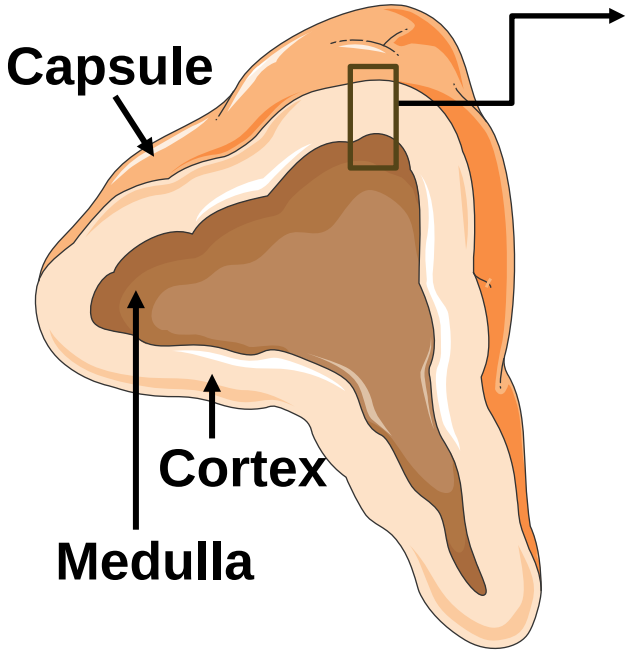
- Introduction
- Anatomy and Histology
- Proliferative Lesions of the Adrenal Cortex
  - **Hyperplastic Lesions**
    - Hyperplasia, cortex, diffuse
    - Hyperplasia, cortex, focal
    - Hyperplasia, subcapsular cell
  - **Benign Tumors**
    - Adenoma, cortex
    - Adenoma, subcapsular cell
  - **Malignant Tumors**
    - Carcinoma, cortex
    - Carcinoma, subcapsular cell
- Proliferative Lesions of the Adrenal Medulla
  - **Hyperplastic Lesions**
    - Hyperplasia, medulla, diffuse
    - Hyperplasia, medulla, focal
  - **Benign Tumors**
    - Pheochromocytoma, benign
    - Pheochromocytoma, complex, benign
  - **Malignant Tumors**
    - Pheochromocytoma, malignant
    - Pheochromocytoma, complex, malignant
- Summary

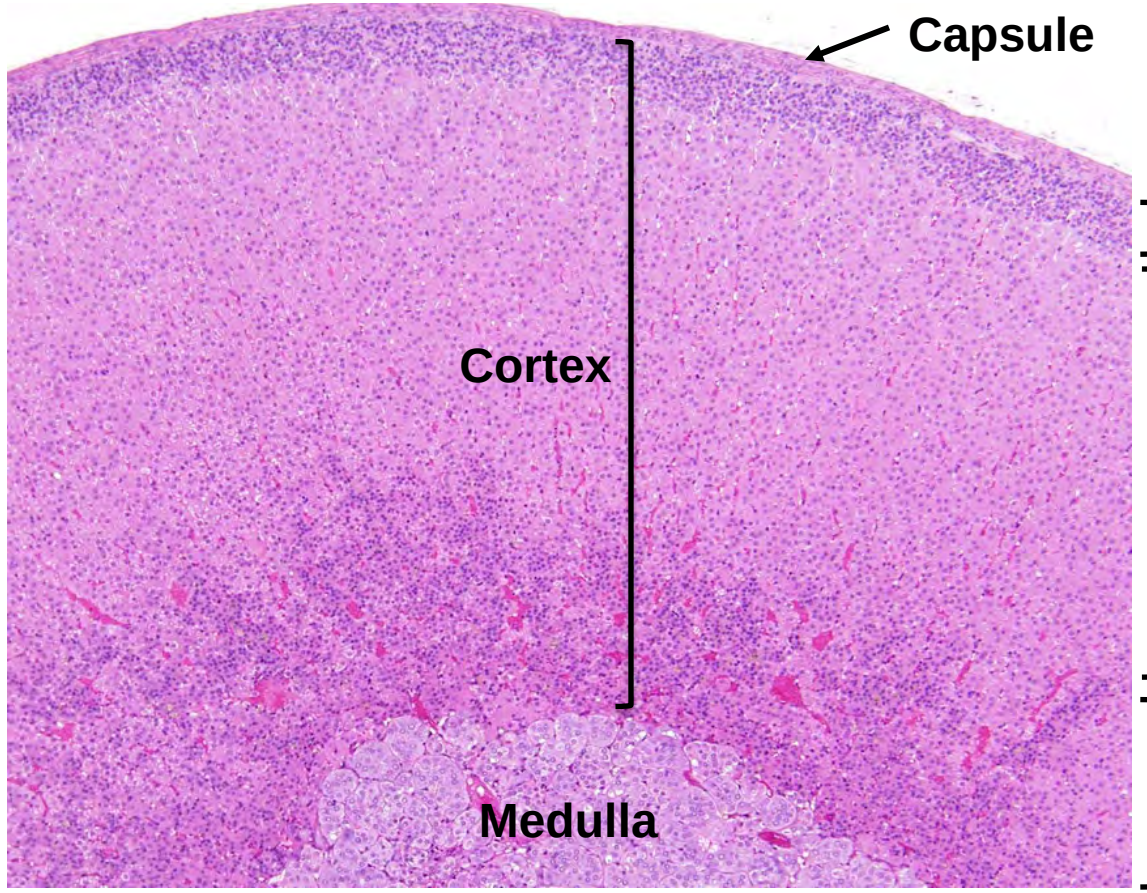
Adrenal Gland – Introduction

- The adrenal gland is composed of portions: the outer cortex and the inner medulla
  - Although embryologically distinct, the two portions of the adrenal gland are functionally related
  - The cortex is essential for life due to its role in the production of steroid hormones, the function of the medulla is not
- Adrenal glands are small, paired, yellowish organs located in the retroperitoneal space at the anterior pole of the kidneys
- In mice, growth and function of the adrenal glands are markedly influenced by sex and age
- In mice and rats, the adrenal gland of the female is significantly larger than that of the male
  - Relative difference varies among different strains
  - Adult female rats generally demonstrate increased sizes for all 3 zones of the adrenal cortex
- Medullary cells are basically modified postganglionic cells of the autonomic nervous system and can be considered a ganglion of the sympathetic nervous system

Adrenal Gland – Histology

Histology (Rat)





Zona glomerulosa	→	Mineralocorticoids (Aldosterone)
Zona fasciculata	→	Glucocorticoids (Cortisol, corticosterone)
Zona reticularis	→	Androgens (Dehydroepiandro-sterone, DHEA)
Adrenal medulla	→	Catecholamines (Epinephrine, Norepinephrine)

Proliferative Lesions of the Adrenal Gland: Introduction

- Adrenal gland cortical and medullary hyperplastic lesions can be spontaneous, age-related changes, or may be related to test article administration
- Hyperplastic lesions consist of localized increases in cortical and/or medullary cell numbers
- Medullary hyperplasia and neoplasia arise in and are confined to the medulla when small but can extend to the cortex and beyond when malignant
- Based on site of origin, there are two diagnostic categories of cortical proliferative lesions: **subcapsular** and **cortical**
  - Subcapsular hyperplasia can progress to benign and malignant neoplasms
  - Cortical hyperplasia in the zona fasciculata infrequently progresses to neoplasms

Proliferative Lesions of the Adrenal Cortex: Background

- Cortical hyperplasia is generally focal, though diffuse hyperplasia may occur
- May be a regenerative response to degeneration and necrosis, the result of increased adrenocorticotrophic hormone (ACTH) secretion by the pituitary, or of unknown cause
- Hormonal manipulation, chemicals, and irradiation have been found to induce proliferative lesions of the cortex
- Focal hyperplasia, adenoma, and carcinoma comprise a morphological continuum
  - It is sometimes difficult to distinguish marked hyperplasia from adenoma and adenoma from carcinoma

Proliferative Lesions of the Adrenal Cortex

- **Hyperplastic Lesions**
  - Hyperplasia, cortex, diffuse
  - Hyperplasia, cortex, focal
  - Hyperplasia, subcapsular cell
- **Benign Tumors**
  - Adenoma, cortex
  - Adenoma, subcapsular cell
- **Malignant Tumors**
  - Carcinoma, cortex
  - Carcinoma, subcapsular cell

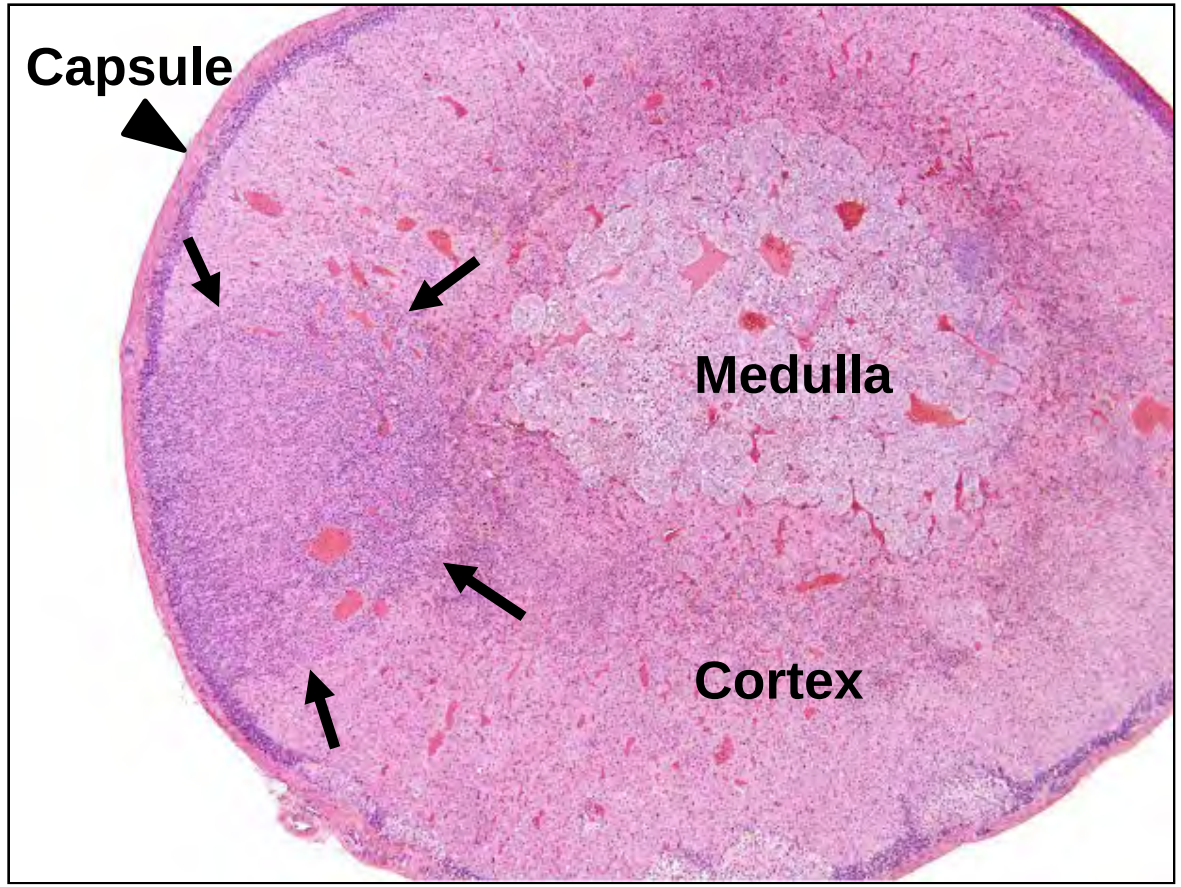
Adrenal Gland – Hyperplasia, Cortex, Diffuse

- Characterized by a diffuse increase in the number of adrenocortical cells in one or more cortical zones
- Accompanied by increases in overall adrenal size, adrenal weight, and thickness of the cortex
- Cortical cells may be normal in size or enlarged (hypertrophy), cytoplasm may be eosinophilic with reduced cytoplasmic vacuoles, finely granular, or filled with small lipid vacuoles (microvesicular)
- Can represent a physiological response to increased serum ACTH (e.g., chronic stress), pregnancy in rats, or a change induced by xenobiotics
- Observed in both mice and rats
- Differential diagnoses include Hypertrophy, cortex, diffuse; and Vacuolation, cortex, increased, diffuse (*see nonproliferative module*)

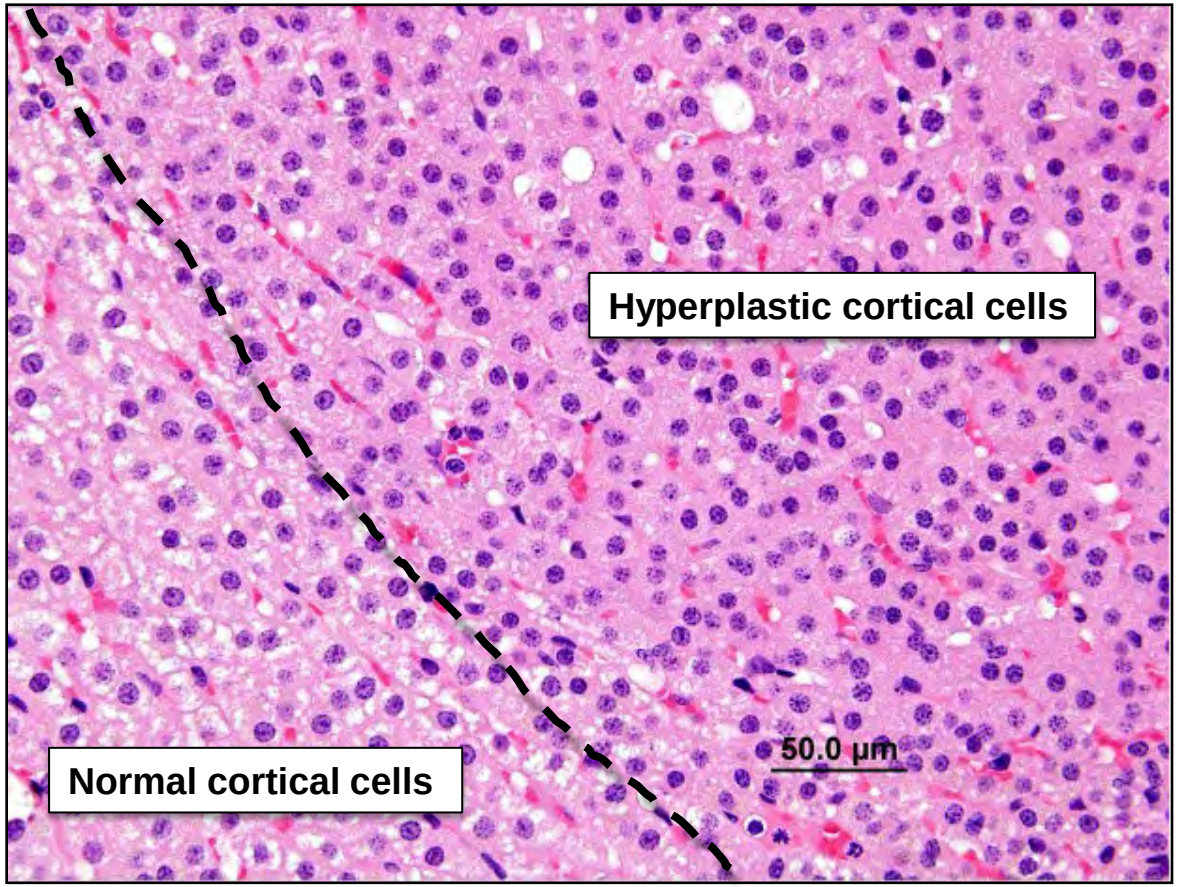
Adrenal Gland – Hyperplasia, Cortex, Focal

- Derived from cells of the adrenal cortex
- Diagnostic features
  - Circumscribed focal or multifocal increase in cell number of the adrenal cortex with no to minimal compression of the surrounding cortex
    - The hyperplastic cells may be smaller or larger than surrounding cortical cells
    - Usually located in the zona fasciculata, but may occur in any cortical zone
  - Hyperplastic cortical cells may have similar cytoplasm as the surrounding cells or may be more basophilic, eosinophilic, or amphophilic; may be vacuolated, especially in the inner part of the focus
  - Usually no cellular pleomorphism, but atypia, karyomegaly or polyploidy may be present
- Differential diagnoses include Adenoma, cortex; Hyperplasia, subcapsular cell (mouse); Hyperplasia, medulla, focal; Vacuolation, cortex, increased, focal (*see nonproliferative module*); Vacuolation, cortex, decreased, focal (see *nonproliferative module*) and Degeneration, cystic
- Observed in both mice and rats

Adrenal Gland – Hyperplasia, Cortex, Focal



A well circumscribed focus of hyperplasia (arrows) without compression of the adjacent cortical tissue in a rat.

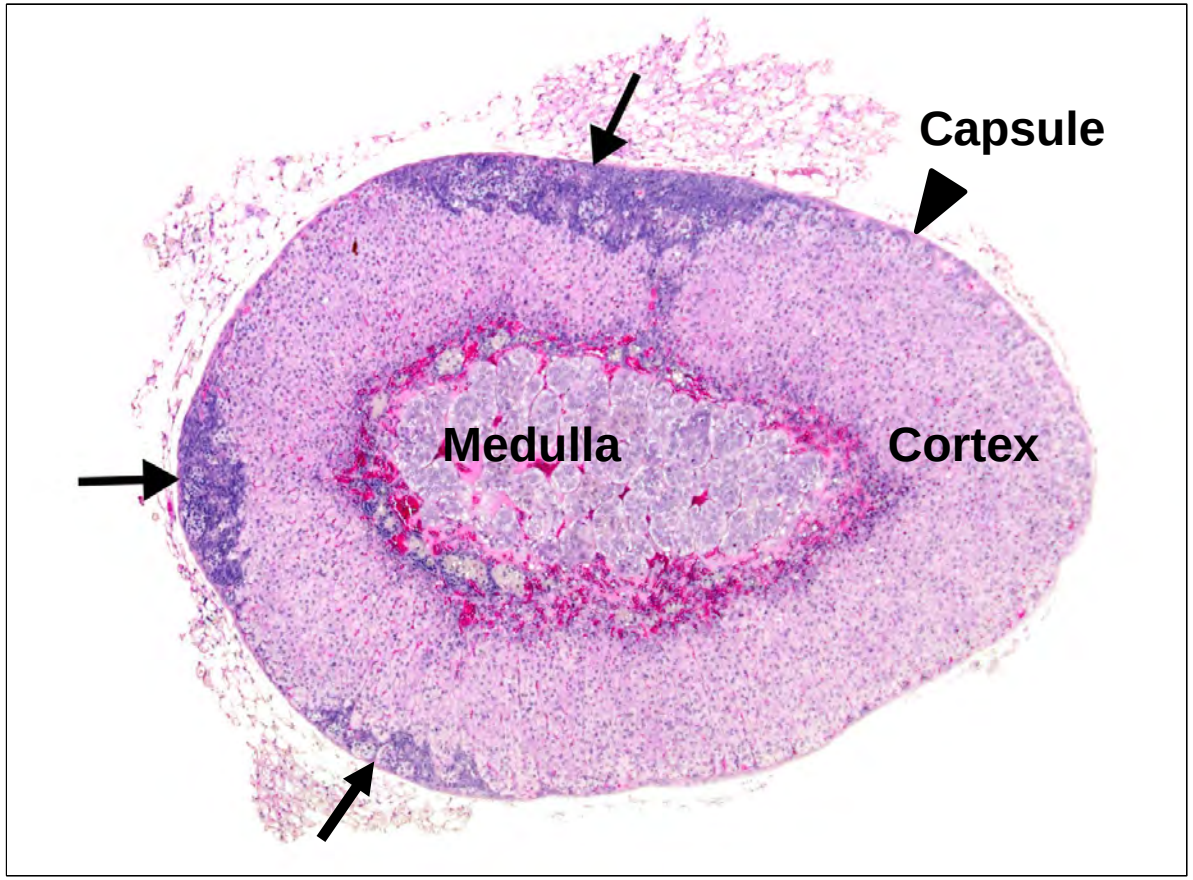


Hyperplastic cortical cells (right side of the dashed line) are increased in number but smaller in size than the adjacent normal cortical cells (left side of the dashed line). Hyperplastic cells may differ tinctorially (eosinophilic, basophilic, as in this case, or amphophilic) from surrounding cells.

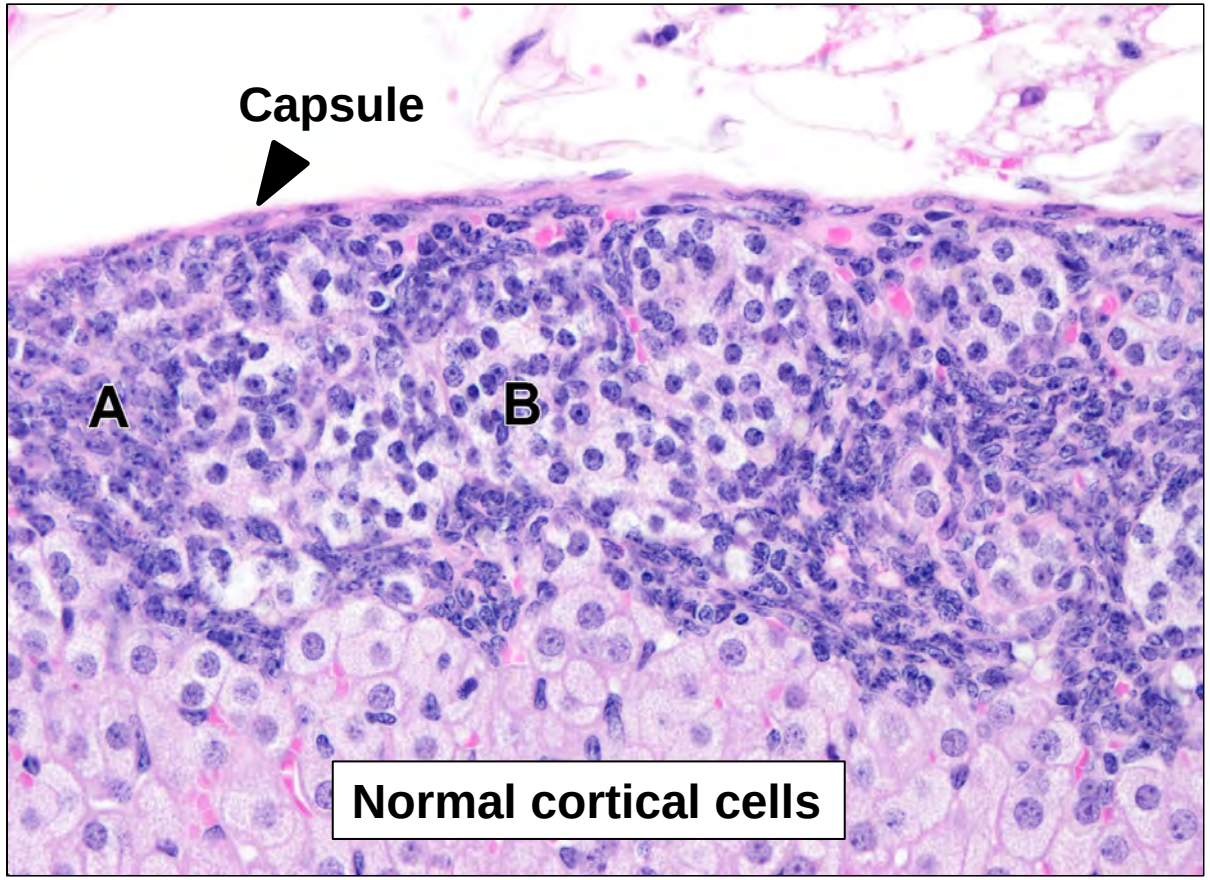
Adrenal Gland – Hyperplasia, Subcapsular Cell

- Derived from undifferentiated progenitor cells in the subcapsular region of the adrenal cortex
- Only present in mice
  - Very common spontaneous finding in older mice of most strains
  - Incidences and severity generally increase with age
  - Much more common and typically more extensive in female mice than in male mice
  - Can be induced in certain mouse strains by gonadectomy and hormonal alterations
- Proliferative cells consist of two types:
  - Type A cells are densely cellular, small, oval to spindle-shaped with scant basophilic cytoplasm and are free of lipid vacuoles; type B cells are large and polygonal with clear cytoplasm and small lipid vacuoles
  - Type A and type B cells are intermingled and the descriptive modifier used is based on the predominant cell type (>70%; i.e., modifiers “Type A” or “Type B”).
  - Early-stage subcapsular hyperplasia is composed primarily of smaller, more basophilic, fusiform, type A (spindle) cells. As the hyperplastic lesions enlarge, type B (polygonal) cells appear, and with further progression the ratio of polygonal type B cells to spindle type A cells usually increases
  - The mixed type of hyperplasia consists of both A and B cells in similar proportions and is the most common type of hyperplasia (modifier “Mixed type”).
- Can be focal, multifocal to circumferential but does not grow into or beyond the capsule, but may bulge from the surface
  - No or minimal compression of the underlying cortex, often wedge-shaped and located beneath the capsule
  - The edge of larger hyperplastic foci can reach the cortico-medullary junction
- Differential diagnoses: Hyperplasia, cortex, focal; Adenoma, cortex

Adrenal Gland – Hyperplasia, Subcapsular Cell



Multiple foci of basophilic hyperplastic cells (arrows) are present in the subcapsular region of the cortex in a mouse.

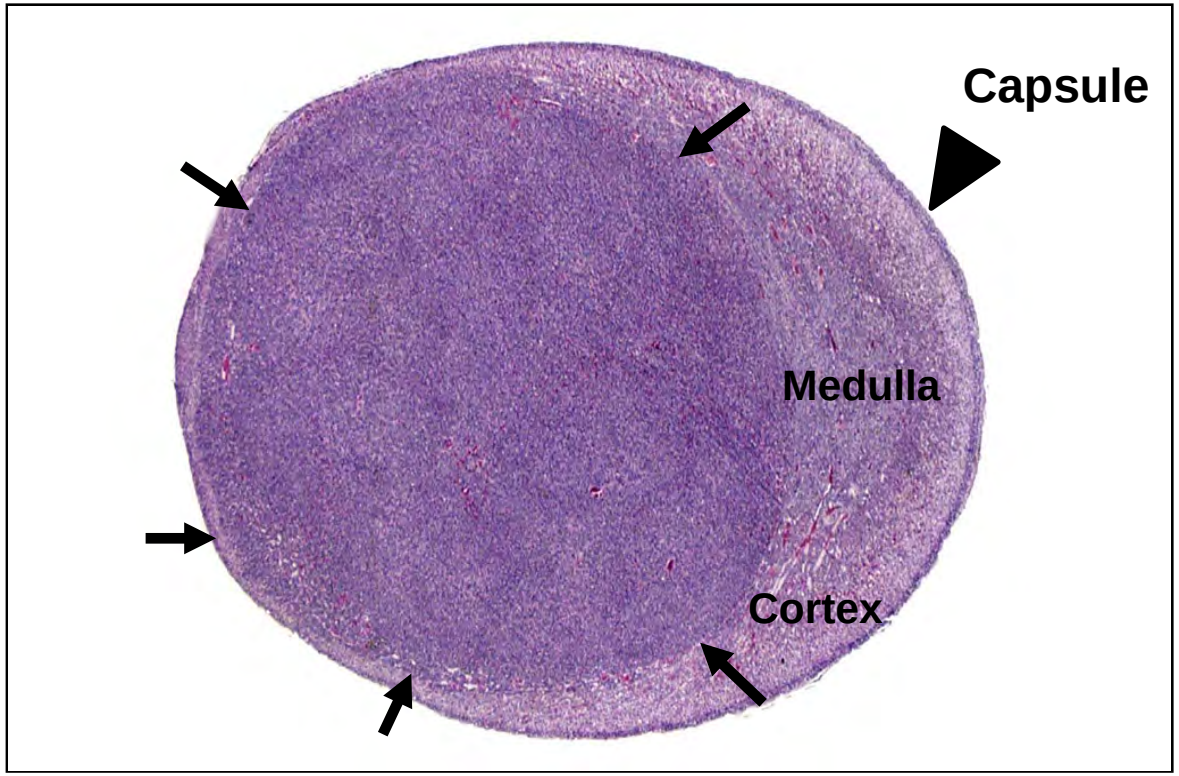


Hyperplastic focus shown here is composed of smaller, fusiform Type A cells with scant basophilic cytoplasm mixed with nests of larger, paler Type B cells with clear cytoplasm.

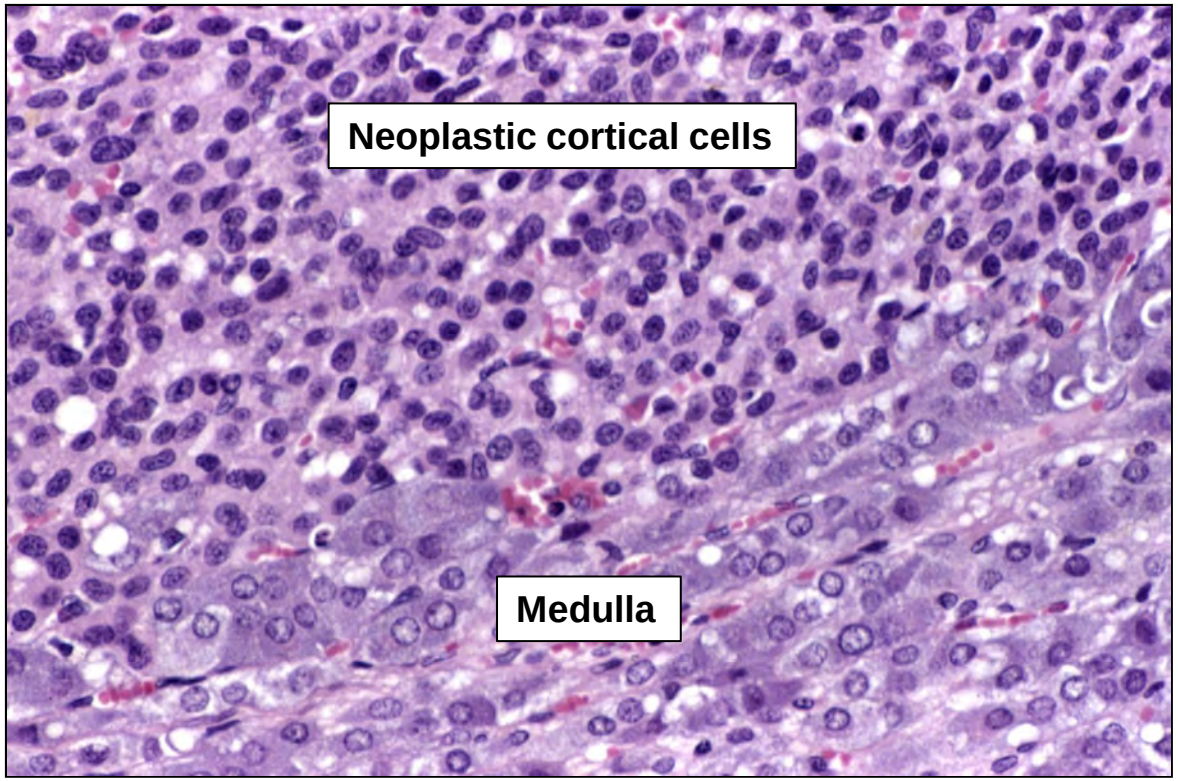
Adrenal Gland – Adenoma, Cortex

- Benign neoplasm derived from adrenocortical cells
- Diagnostic features
  - Well-demarcated nodule or mass of cells that may have a thin capsule, with distinct compression of adjacent parenchyma (cortical and/or medullary tissue)
  - Normal architecture is not maintained: cells are arranged in cords, trabeculae, or solid clusters
  - Rats: cells may be smaller or enlarged and may exhibit tinctorial differences
  - Mice (rare in mice): usually composed of enlarged, eosinophilic or amphophilic cells
  - Mitotic figures and cellular atypia may be present
  - Other features may be present: vacuolation, angiectasis (dilated vessels), hemorrhage (blood outside vessels), thrombosis (clot)
- Differential diagnoses: Hyperplasia, cortex, focal; Adenoma, subcapsular cell (mouse); Carcinoma, cortex; Pheochromocytoma, benign

Adrenal Gland – Adenoma, Cortex



A well circumscribed adrenal cortex adenoma (arrows) from a rat, with distinct compression of the adjacent cortical and medullary tissues.

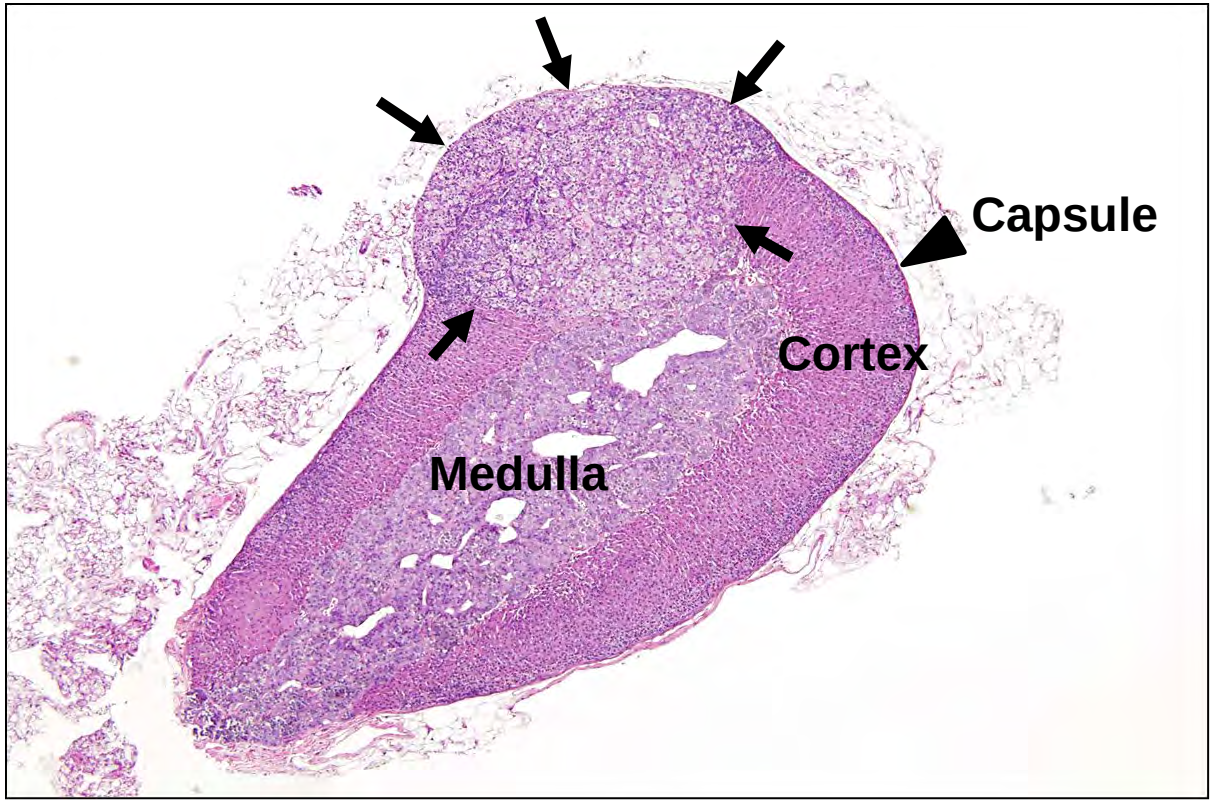


The neoplastic cells are smaller in size and appear more basophilic than the rest of the cortex, and the adenoma compresses cells of the medulla.

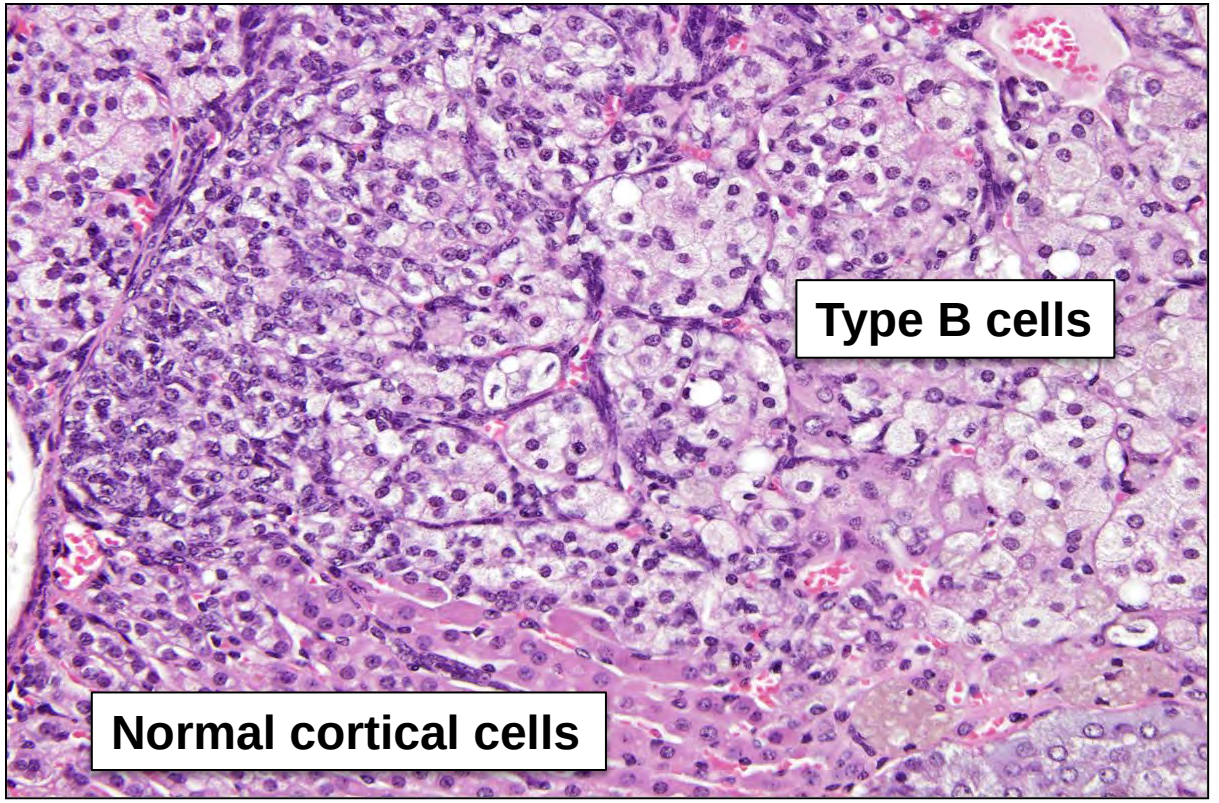
Adrenal Gland – Adenoma, Subcapsular Cell

- Benign neoplasm derived from undifferentiated progenitor cells in the subcapsular region of the adrenal cortex
- Diagnostic features
  - Mass that bulges from the surface of the adrenal gland and occurs in the subcapsular region of the cortex
  - Mild to marked compression of the underlying cortex
  - Mitotic figures are rare
- Proliferative cells consist of two types
  - Type A cells are densely cellular, small or oval with small amount of basophilic cytoplasm and free of lipid vacuoles
  - Type B cells are large and polygonal with clear cytoplasm and small lipid vacuoles
  - The descriptive modifier used is based on the predominant cell type (>70%); mixed type is most common and has similar proportions of Type A and Type B cells
  - May arise as a distinct Type B cell proliferation within subcapsular cell hyperplasia; subcapsular adenomas, especially those of Type B cells, may be hormonally active, with estrogenic, androgenic, or adrenocorticoid effects
- Only present in mice
- Differential diagnoses: Hyperplasia, subcapsular cell; Carcinoma, subcapsular cell; Adenoma, cortex

Adrenal Gland – Adenoma, Subcapsular Cell, Type B



A well-circumscribed subcapsular adenoma (arrows) in a mouse, consisting mostly of Type B cells.

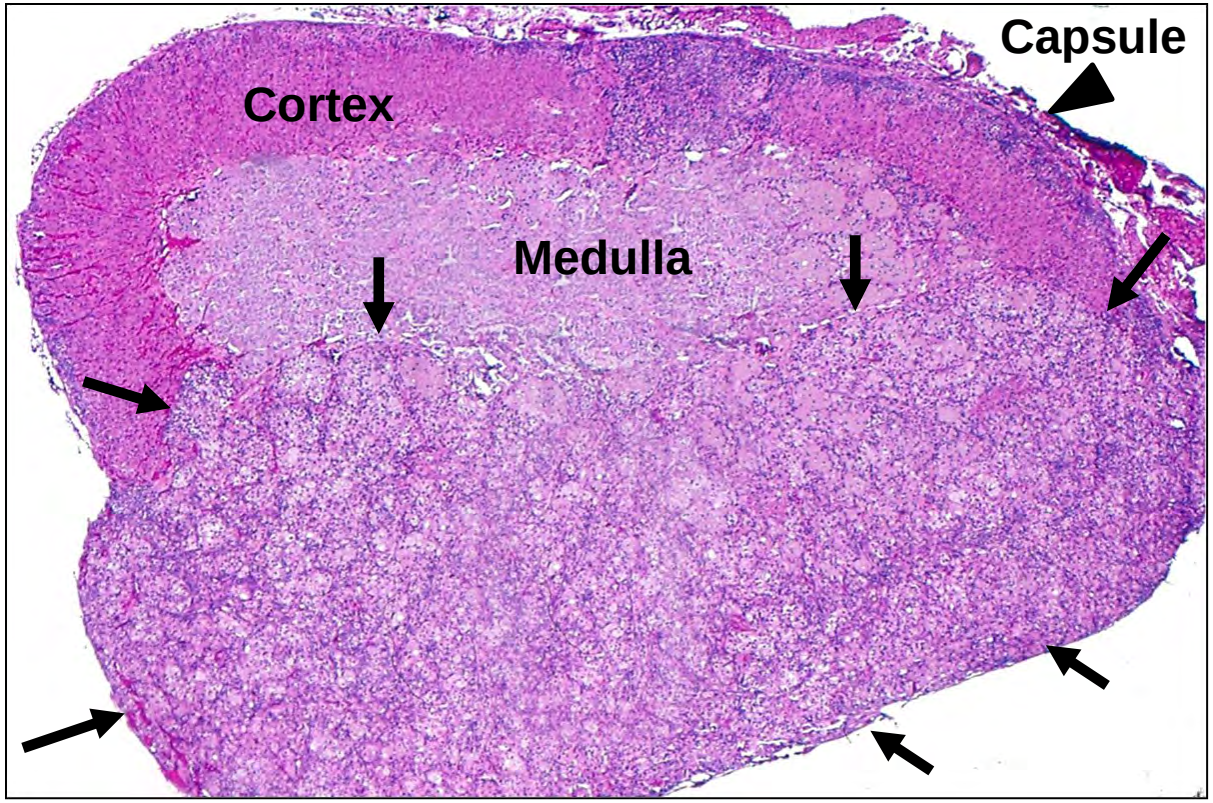


Neoplastic cells in this adenoma are Type B cells: polygonal, pale cytoplasm, and contain lipid vacuoles.

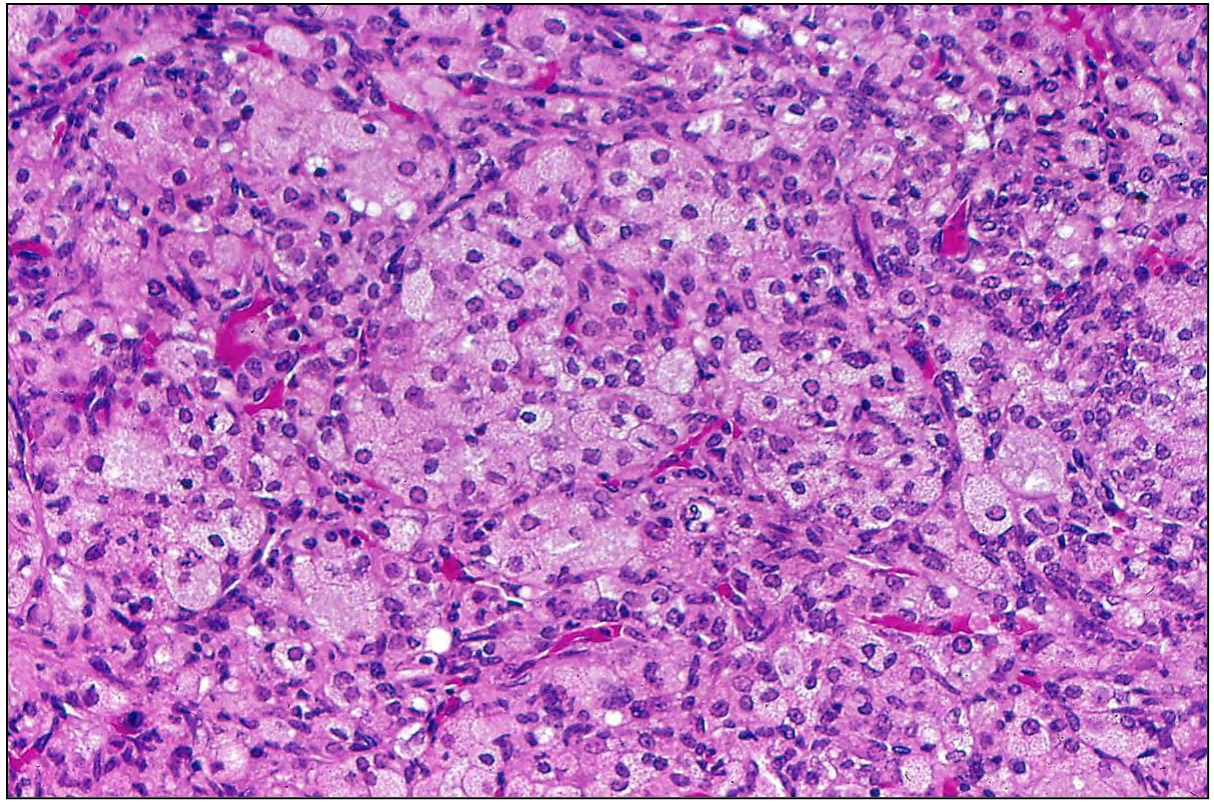
Adrenal Gland – Carcinoma, Cortex

- Malignant neoplasm derived from adrenal cortical cells
- Diagnostic features
  - Large, irregularly shaped mass that invades into surrounding adrenal tissue, beyond the adrenal capsule and/or shows distant metastasis
  - Neoplastic cells are organized in thickened trabeculae, sheets or solid clusters with disruption of normal architecture
  - Usually have eosinophilic or amphophilic cytoplasm
  - Often show pleomorphism (variation in size and shape of cells and/or nuclei)
  - Vacuolation, cystic degeneration, necrosis, angiectasis, or hemorrhage may be present
  - May have numerous mitotic figures
- Carcinomas usually efface the normal architecture (cellular pattern and zonation), distort or displace the medulla, and invade the capsule and adjacent soft tissues or blood vessels
- Differential diagnoses: Carcinoma, subcapsular cell (mice); Adenoma, cortex; Pheochromocytoma, malignant
- Observed in rats and mice, though rare in mice

Adrenal Gland – Carcinoma, Cortex



A large, irregularly shaped cortical carcinoma (arrows) from a mouse effaces and invades the adrenal cortex and medulla.

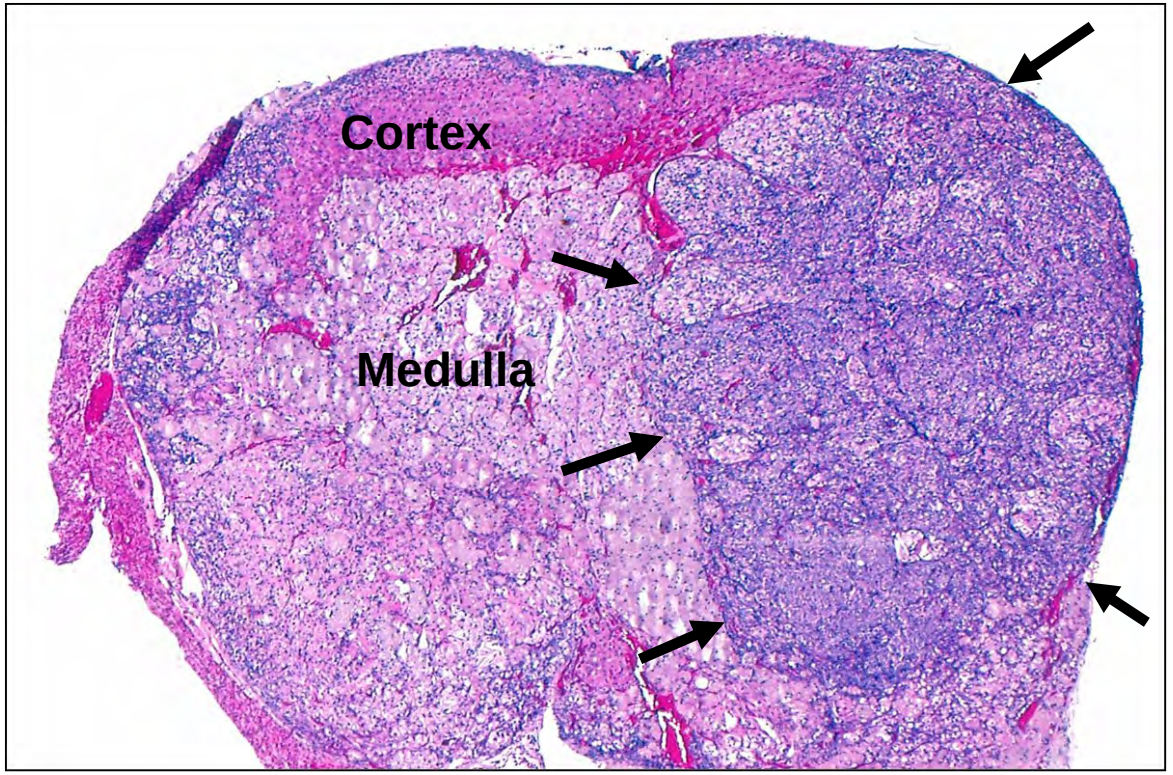


Neoplastic cells in this carcinoma are pleomorphic and have amphophilic cytoplasm.

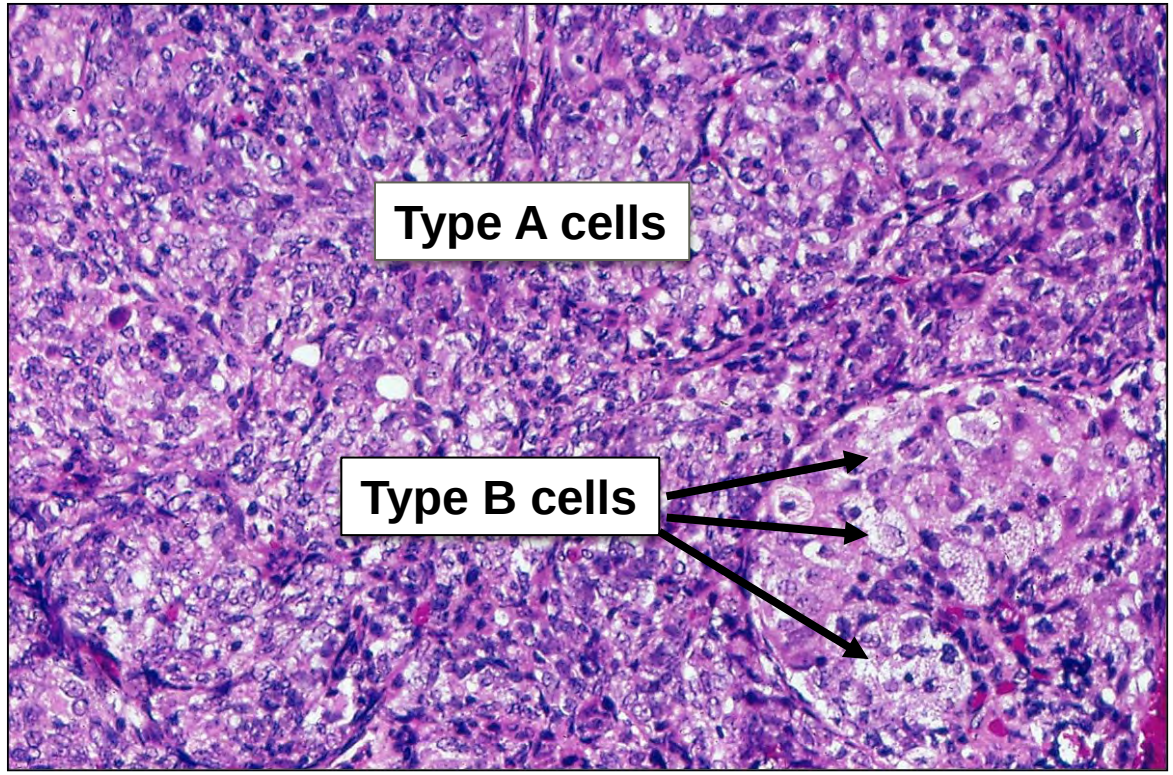
Adrenal Gland – Carcinoma, Subcapsular Cell

- Malignant neoplasm derived from undifferentiated progenitor cells in the subcapsular region of the adrenal cortex
- Diagnostic features
  - Larger tumor than subcapsular adenoma with distinct invasion into the surrounding capsule and adjacent fatty tissue, and/or distant metastases (usually to the lung)
  - Pleomorphism and frequent mitotic figures are characteristic
  - Neoplastic cells often arranged in sheets, although they may be organized in nests, ribbons or cords, or herringbone patterns
- Neoplastic cells consist of two types (Type A or B)
  - The descriptive modifier (“Type A”, “Type B”, “Mixed Type”) used is based on the predominant cell type (>70%).
    - Tumor cells are usually small with oval to fusiform nuclei, resembling Type A cells
    - Metastases have been reported to occur from Type A cells
  - Mixed Type: no predominant cell type is present
- Occurs in mice
- Differential diagnoses: Adenoma, subcapsular cell; Carcinoma, cortex

Adrenal Gland – Carcinoma, Subcapsular Cell



Subcapsular cell carcinoma (arrows) from a mouse, consisting mostly of small, dark Type A cells.



Small nests of Type B cells that are polygonal, pale and contain lipid vacuoles can be seen mixed with small, basophilic, spindle shaped Type A cells.

Proliferative Lesions of the Adrenal Medulla: Background

- Proliferative lesions are observed in both mice and rats
- Incidence of spontaneous proliferative lesions of the adrenal medulla (hyperplasia and pheochromocytoma) varies among rat strains and sex
  - They occur frequently in aging Sprague Dawley, Han Wistar, and Fischer F344 rats
  - More common in males than females
- Lesions rarely occur before one year of age, and the incidence increases thereafter
- Incidence is known to be affected by environmental factors, strain, endocrine conditions, diet, and chemical treatment
- Medullary proliferative lesions are sometimes observed in conjunction with proliferative lesions of the pituitary gland (pars distalis), thyroid C cells, and pancreatic islets

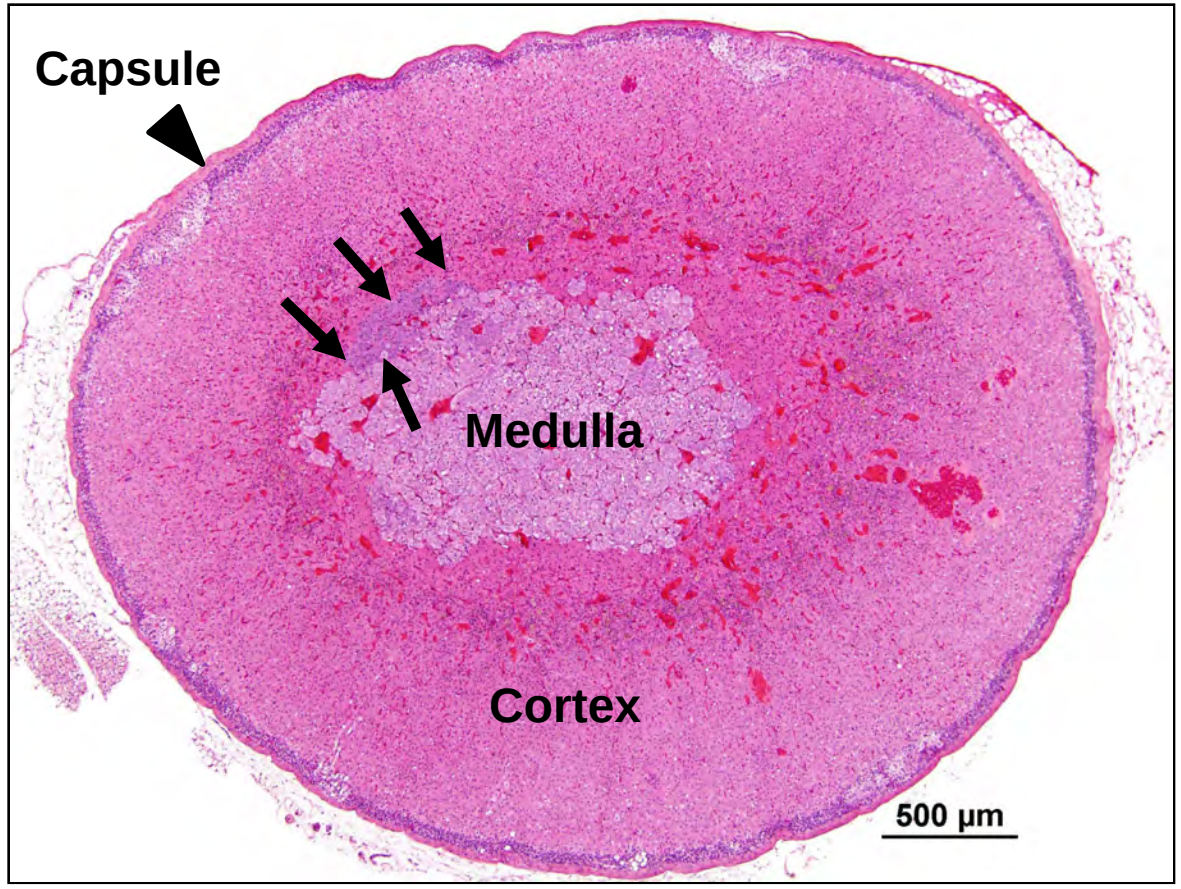
Proliferative Lesions of the Adrenal Medulla

- **Hyperplastic Lesions**
  - Hyperplasia, medulla, diffuse
  - Hyperplasia, medulla, focal
- **Benign Tumors**
  - Pheochromocytoma, benign
  - Pheochromocytoma, complex, benign
- **Malignant Tumors**
  - Pheochromocytoma, malignant
  - Pheochromocytoma, complex, malignant

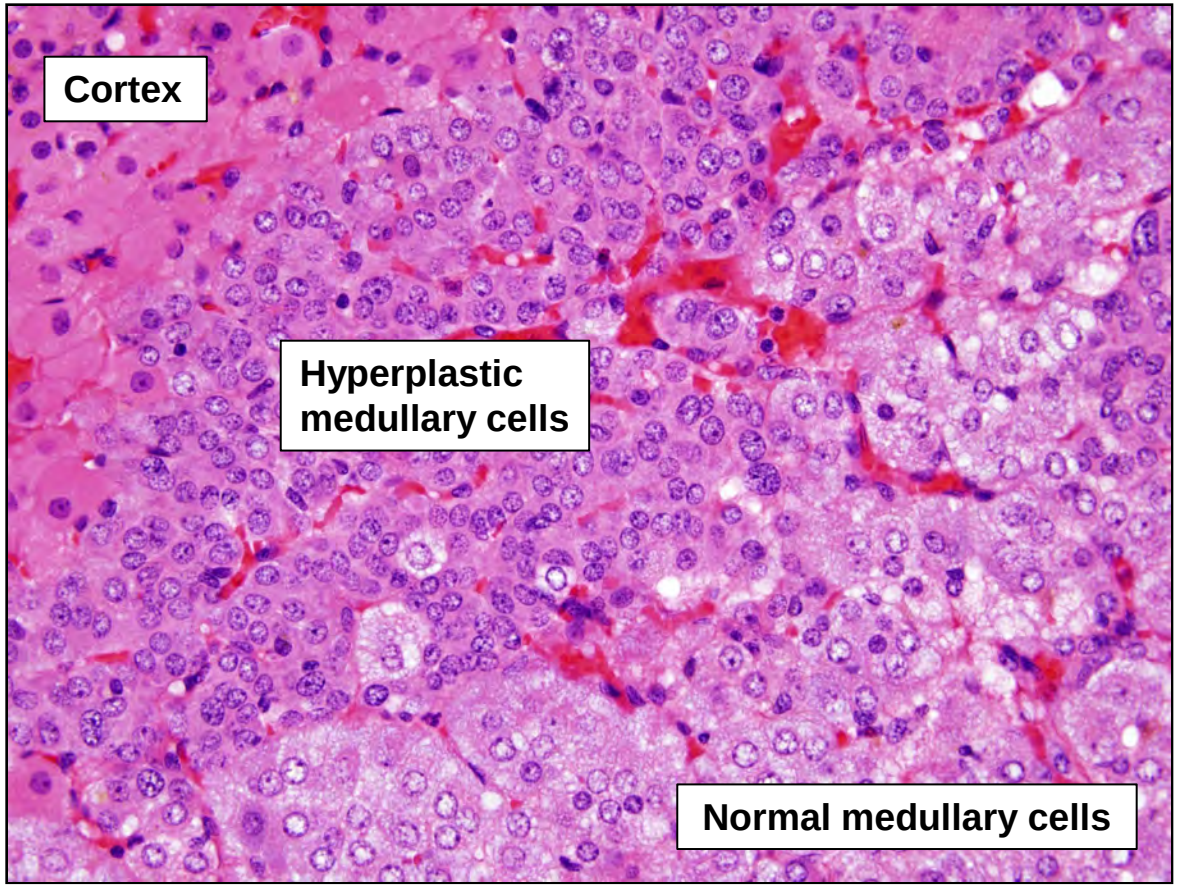
Adrenal Gland – Hyperplasia, Medulla, Focal

- Derived from adrenal medullary, or chromaffin, cells (neuroendocrine cells that release hormones into the blood)
- Diagnostic features
  - Focal, circumscribed increase (usually less than 50% of the normal medulla) in number of chromaffin cells that is usually solitary, infrequently multifocal
  - Cellular architecture is maintained and there is no or minimal compression of medulla or cortex
  - Cell size varies and cells may have tinctorial differences of the cytoplasm, often appearing more basophilic
- Focal hyperplasia may occur anywhere in the medulla but often appears to arise near the corticomedullary junction
- Cells in medullary hyperplasia can consist of both epinephrine (adrenaline) and norepinephrine (noradrenaline) producing cells
- Incidence is strain dependent and increases with age
- Observed in both mice and rats, but is much more common in rats
- Differential diagnoses: Pheochromocytoma, benign

Adrenal Gland – Hyperplasia, Medulla, Focal



A small, noncompressive focus of hyperplastic medullary cells (arrows) is present at the corticomedullary junction in the adrenal gland from a rat.

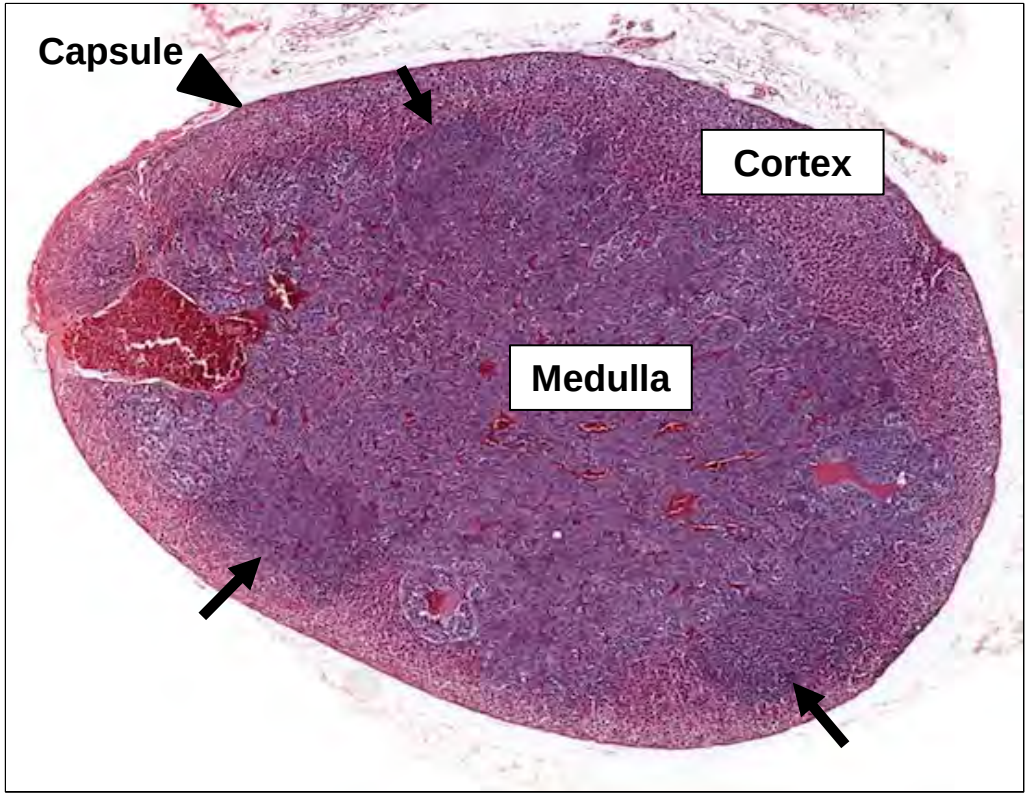


Hyperplastic cells are increased in number but are smaller in size and have more basophilic cytoplasm than the adjacent normal medullary cells.

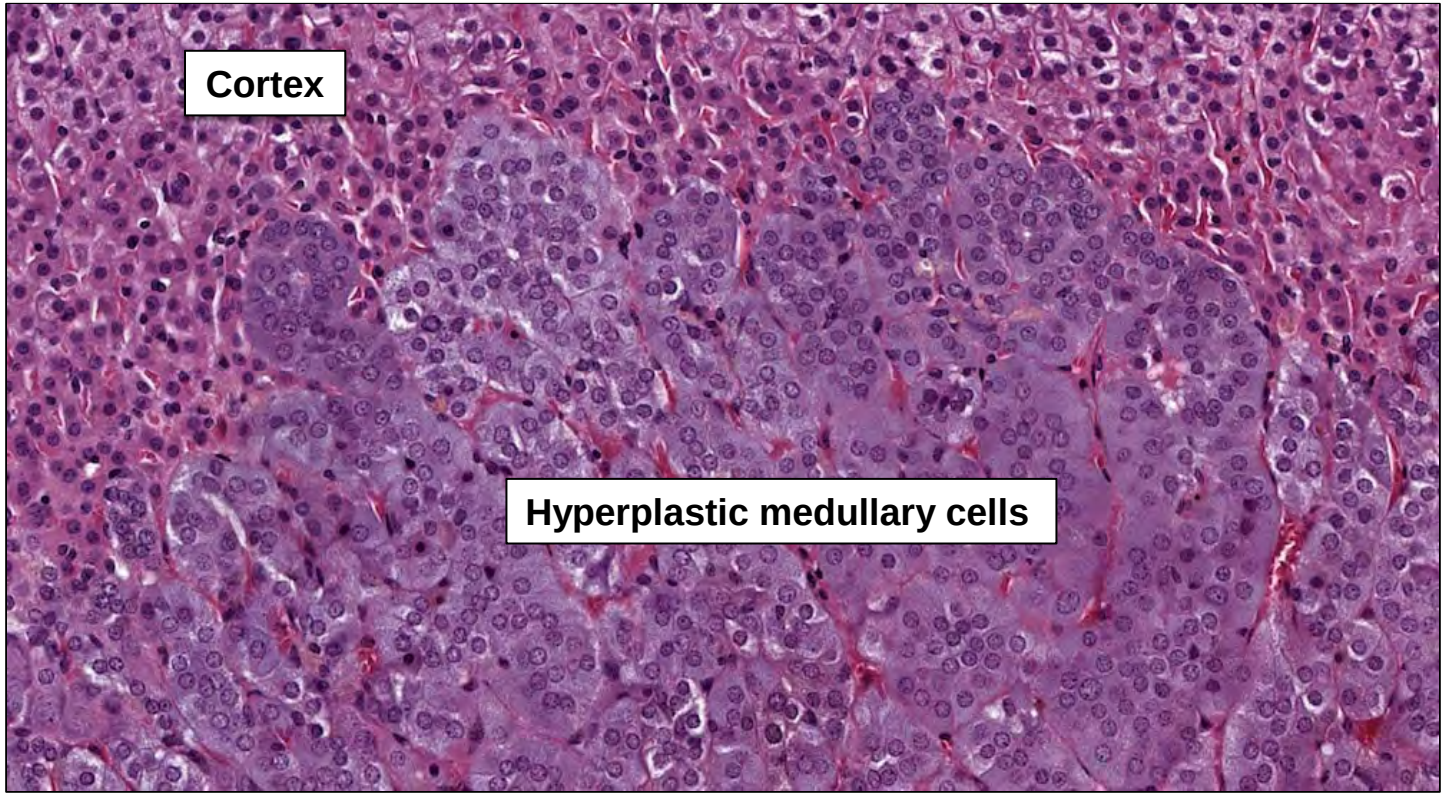
Adrenal Gland – Hyperplasia, Medulla, Diffuse

- Derived from adrenal medullary (chromaffin) cells
- Diagnostic features
  - Medulla is diffusely wider than usual due to increased chromaffin cells, but the architecture is generally maintained, with no or minimal compression of the surrounding cortex
  - Frequently associated with a decreased cortex to medulla ratio
  - Hyperplastic cells may be smaller or larger and have tinctorial differences in the cytoplasm
  - Nuclear to cytoplasm ratio may be increased
- Observed in both mice and rats, but is more common in rats and incidence is strain dependent
  - Rat chromaffin cells respond readily to mitogenic stimulation, in contrast to mice and humans
- Differential diagnosis: Pheochromocytoma, benign

Adrenal Gland – Hyperplasia, Medulla, Diffuse



The adrenal medulla is expanded and distorted by irregular patches of basophilic cells (black arrows) extending into the adrenal cortex in a mouse. The adjacent cortex shows little evidence of compression.



Hyperplastic medullary cells are arranged in small nests of darkly stained compact cells.

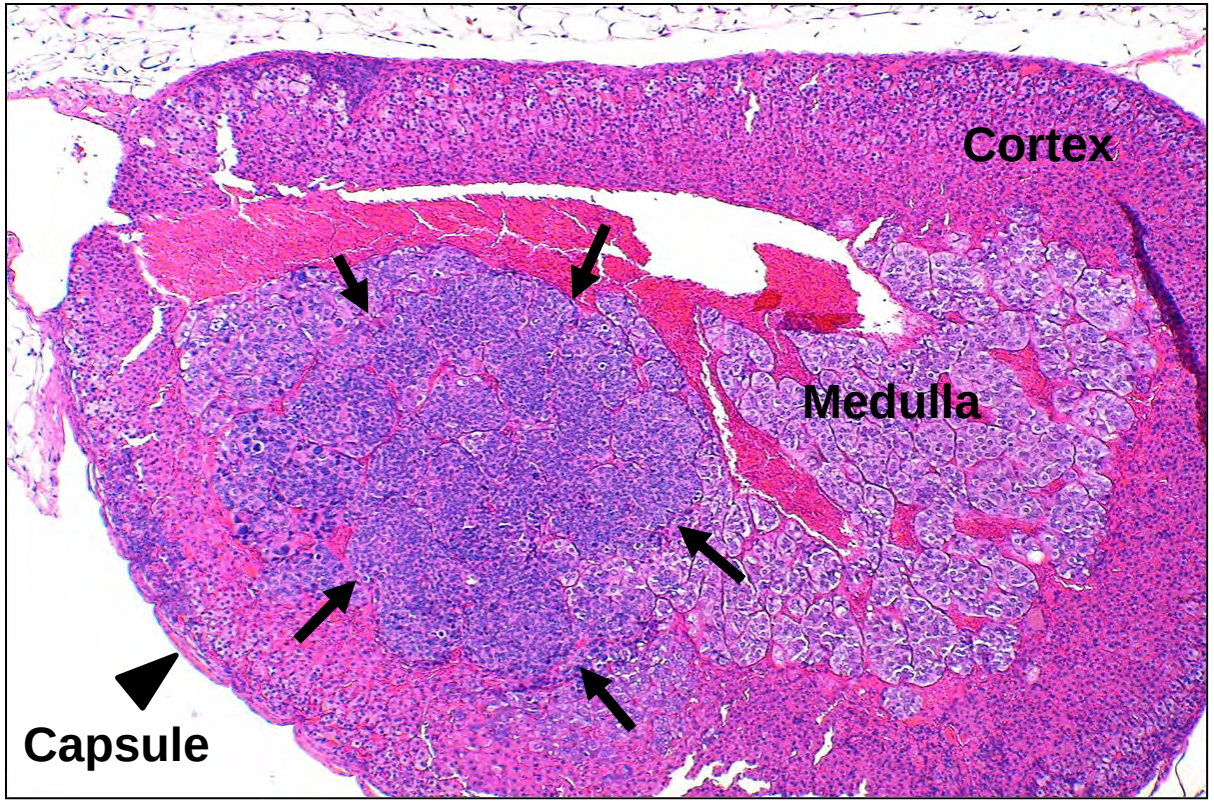
Images from: https://focusontoxpath.com/figures-pathology-of-the-mouse-adrenal-gland/

Adrenal Gland – Pheochromocytoma, Benign

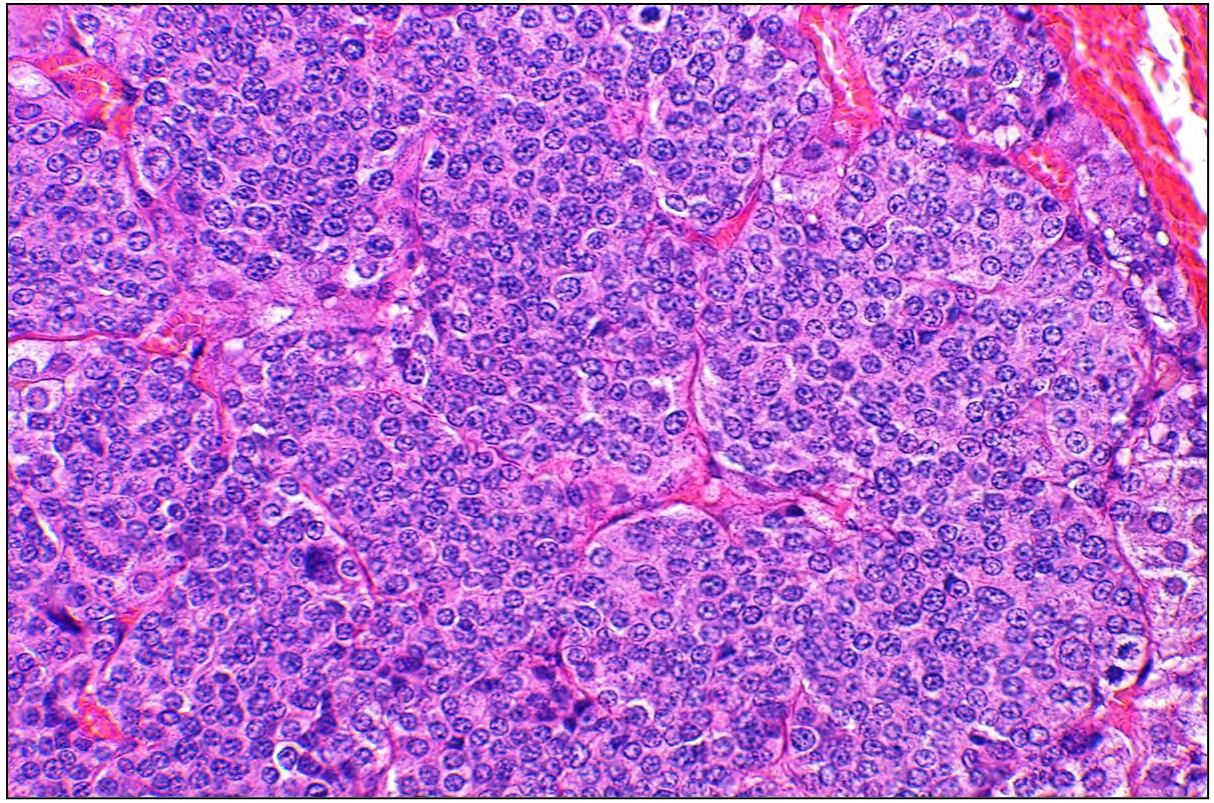
- Benign tumor derived from adrenal medullary (chromaffin) cells
- Diagnostic features
  - Medullary mass usually larger than 50% of normal medulla that may extend into the cortex and replace the entire medulla and cortex
    - Single or multiple
  - Unilateral or bilateral in rats; usually unilateral in the mouse
  - Compression of the normal cortex or medulla at the tumor periphery
  - Neoplastic cells are organized in nests, rows, and cords and mass often contains dilated blood vessels
    - Cell size varies and the smaller cells have greater cytoplasmic basophilia
    - Cellular atypia, hemorrhage and necrosis may be present
- Differential diagnoses: Hyperplasia, medulla, focal; Hyperplasia, medulla, diffuse; Pheochromocytoma, complex, benign; Pheochromocytoma, malignant; Adenoma, cortex

National Institute of Environmental Health Sciences
Division of Translational Toxicology

Adrenal Gland – Pheochromocytoma, Benign



A benign pheochromocytoma filling the adrenal medulla of a mouse (arrows). The cells of the pheochromocytoma have greater cytoplasmic basophilia as compared to the normal medullary cells.

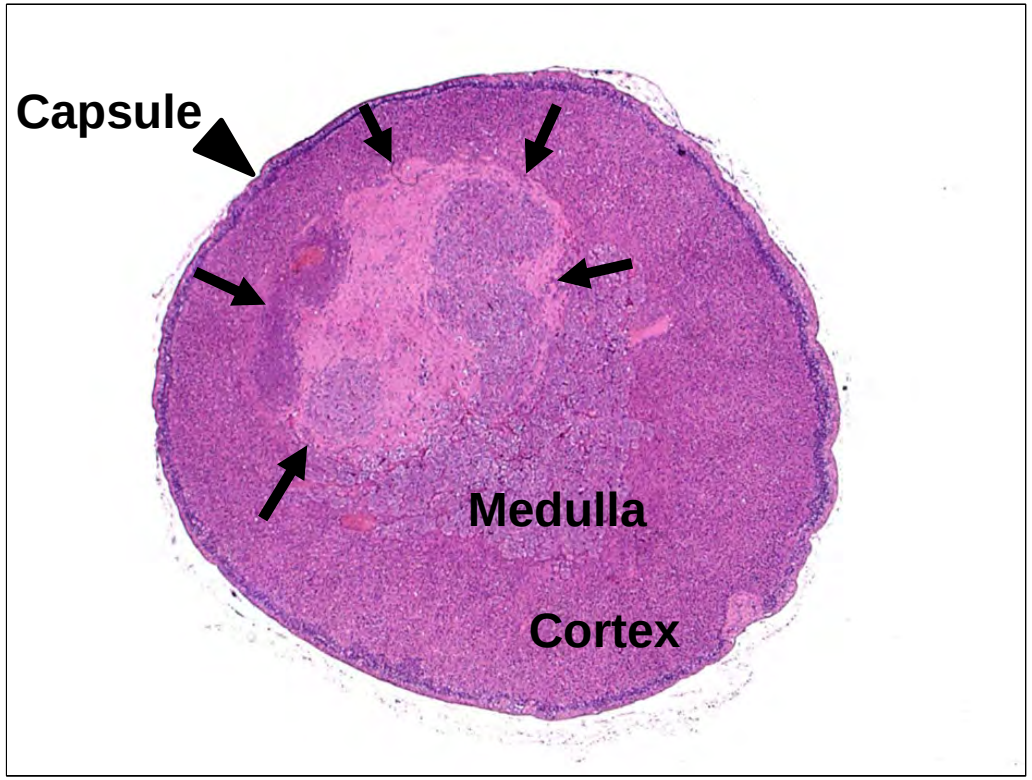


The tumor is composed of nests of uniform neoplastic cells.

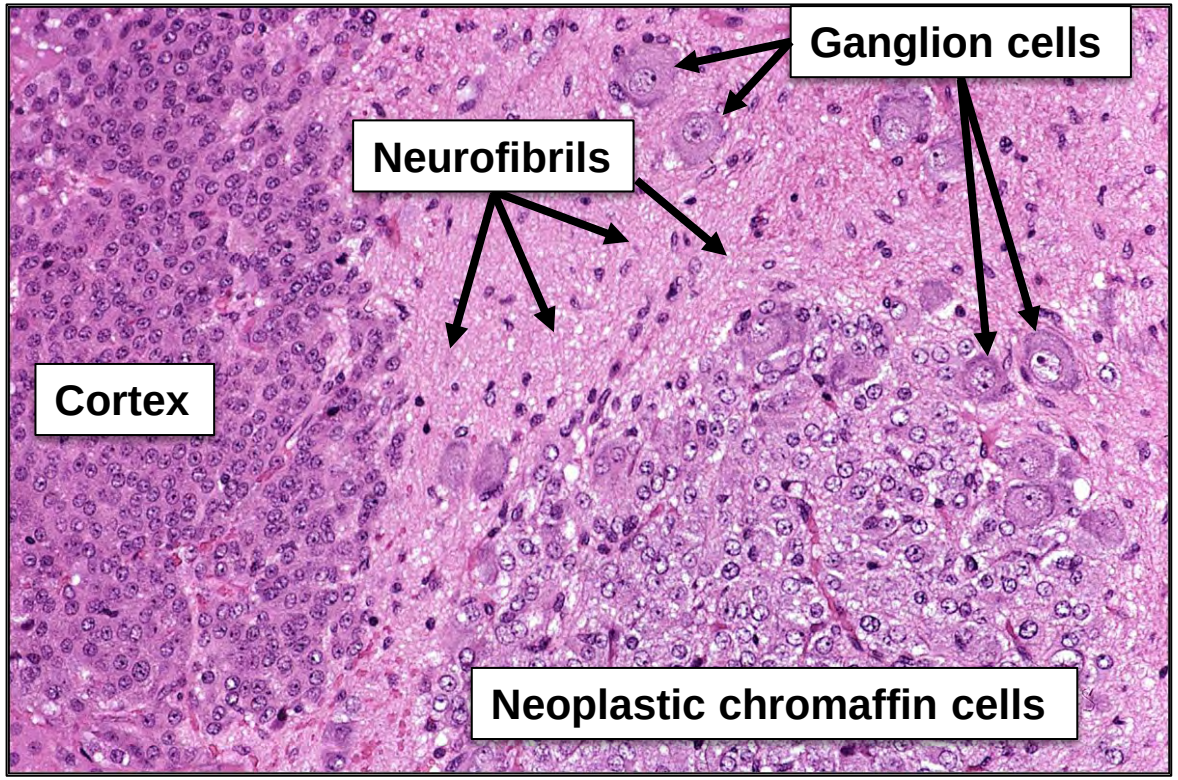
Adrenal Gland – Pheochromocytoma, Complex, Benign

- Benign tumor derived from adrenal medullary (chromaffin) cells
- Diagnostic features
  - Contains morphological components of both pheochromocytoma and ganglioneuroma, but neither component predominates (i.e., neither component comprises >80% of the mass)
  - Cell types include chromaffin cells and well-differentiated ganglion cells with eosinophilic nerve fibers
  - Cells may be variably sized, with scant basophilic cytoplasm, and may be intermingled with nerve fibers or form distinct areas
  - Located in the adrenal medulla, but may extend into the cortex
  - Distinct compression at the tumor periphery
  - Usually larger than 50% of the normal thickness of the medulla
  - Cellular atypia may be present, and mitotic figures may be absent or infrequent
- Observed in both mice and rats
- Differential diagnoses: Hyperplasia, medulla, focal; Pheochromocytoma, complex, malignant; Pheochromocytoma, malignant; Pheochromocytoma, benign; Ganglioneuroma, benign

Adrenal Gland – Pheochromocytoma, Complex, Benign



A benign complex pheochromocytoma (arrows) from a rat that expands the adrenal medulla and extends into the cortex.

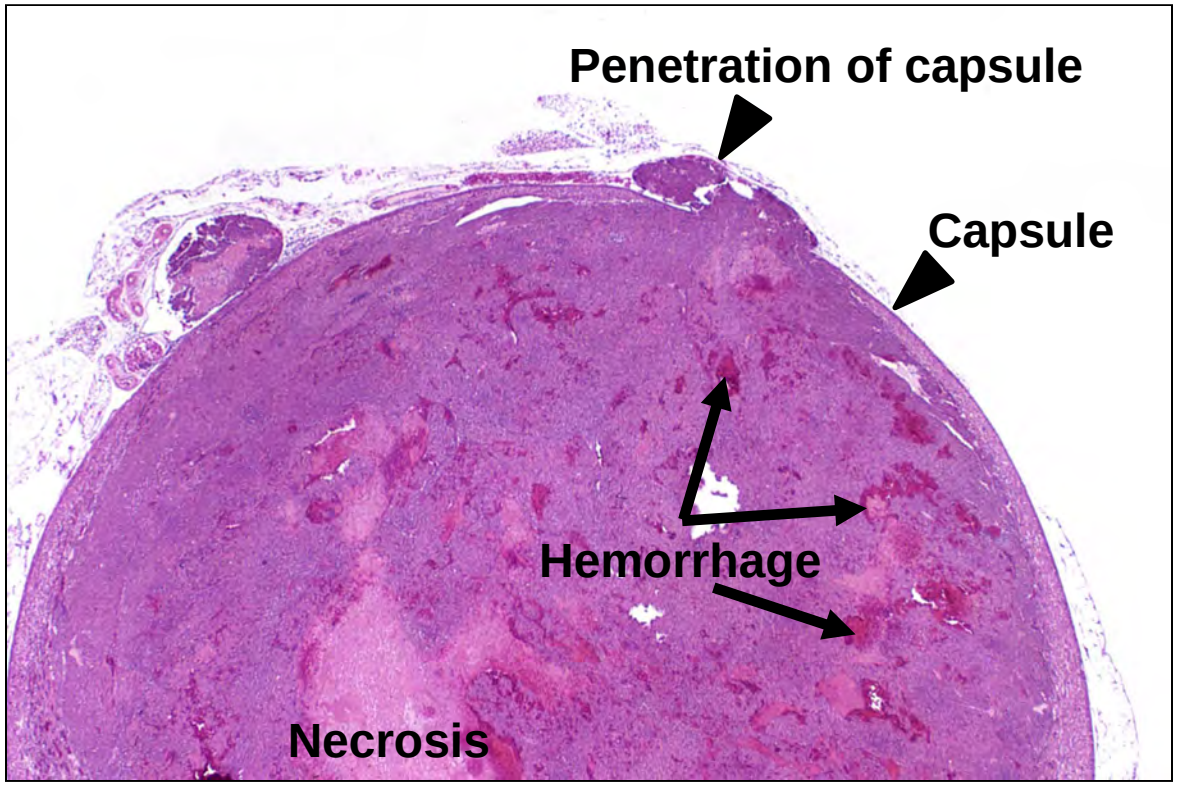


The tumor is composed of well-differentiated ganglion cells, eosinophilic nerve fibers (neurofibrils) and neoplastic chromaffin cells.

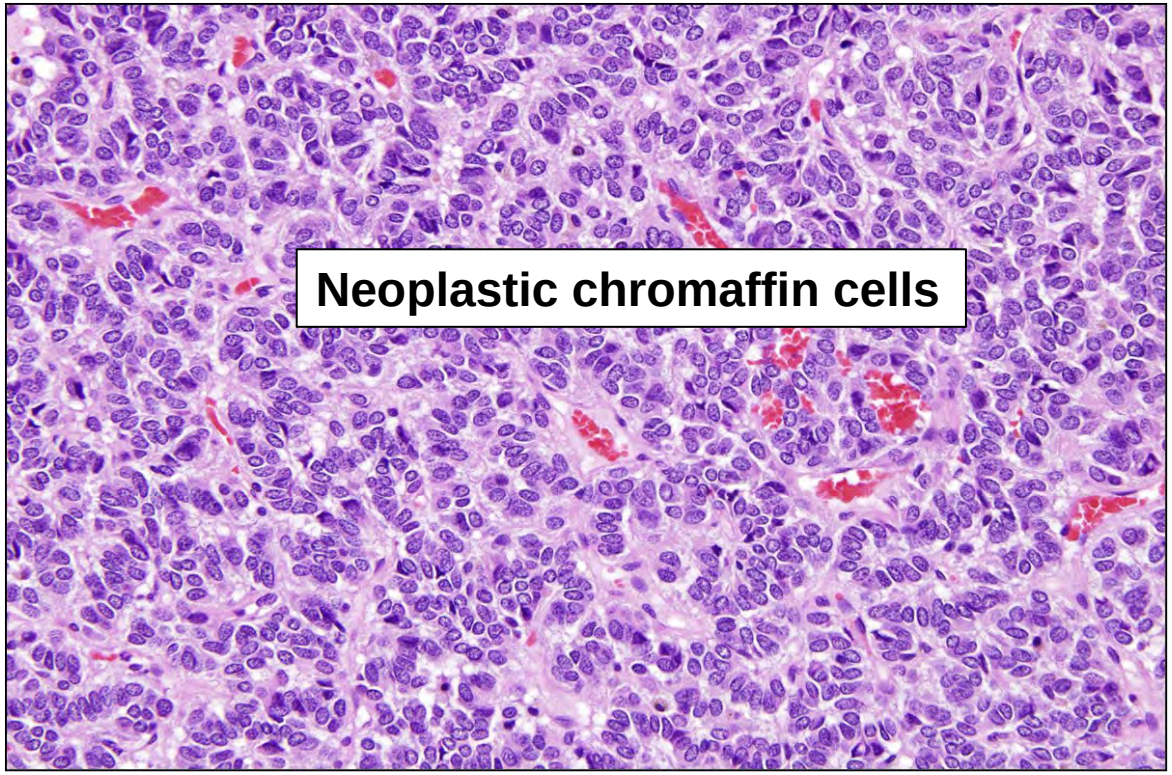
Adrenal Gland – Pheochromocytoma, Malignant

- Malignant tumor derived from adrenal medullary (chromaffin) cells
- Diagnostic features
  - Medullary mass with evidence of malignancy, as indicated by invasive growth into the adrenal cortex or through the adrenal capsule, vascular invasion, or distant metastasis
    - Metastasis occurs most frequently to the lung, liver, and regional lymph nodes
  - Neoplastic cells are organized in nests, rows and cords often with distended blood vessels
  - Cell size varies, with the smaller cells having greater cytoplasmic basophilia
  - Cellular atypia, hemorrhage and necrosis may be present, and mitotic figures may be numerous
- Observed in both mice and rats
- Differential diagnoses: Pheochromocytoma, benign; Carcinoma, cortex; Pheochromocytoma, complex, malignant; Neuroblastoma, malignant

Adrenal Gland – Pheochromocytoma, Malignant



A malignant pheochromocytoma from a rat showing invasion of the cortex, expansion through the capsule, and areas of necrosis (pale pink areas) and hemorrhage (arrows; red areas).

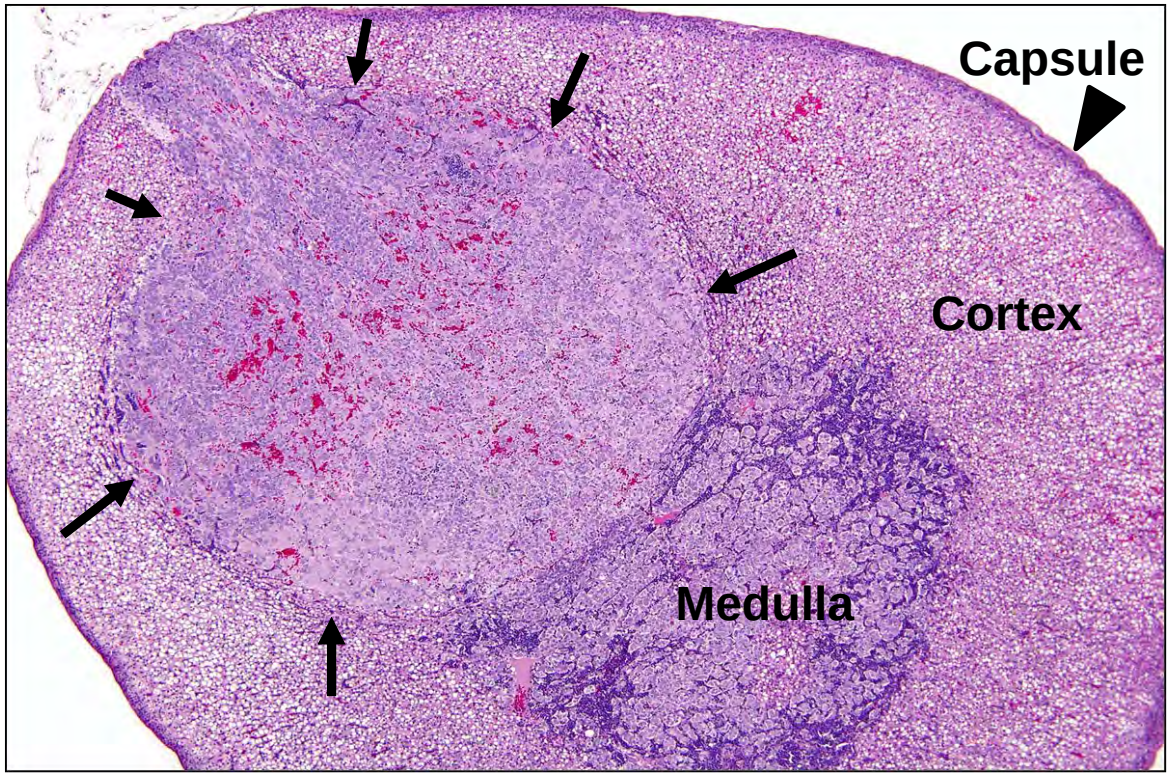


Neoplastic cells are small with hyperchromatic nuclei and are arranged in nests.

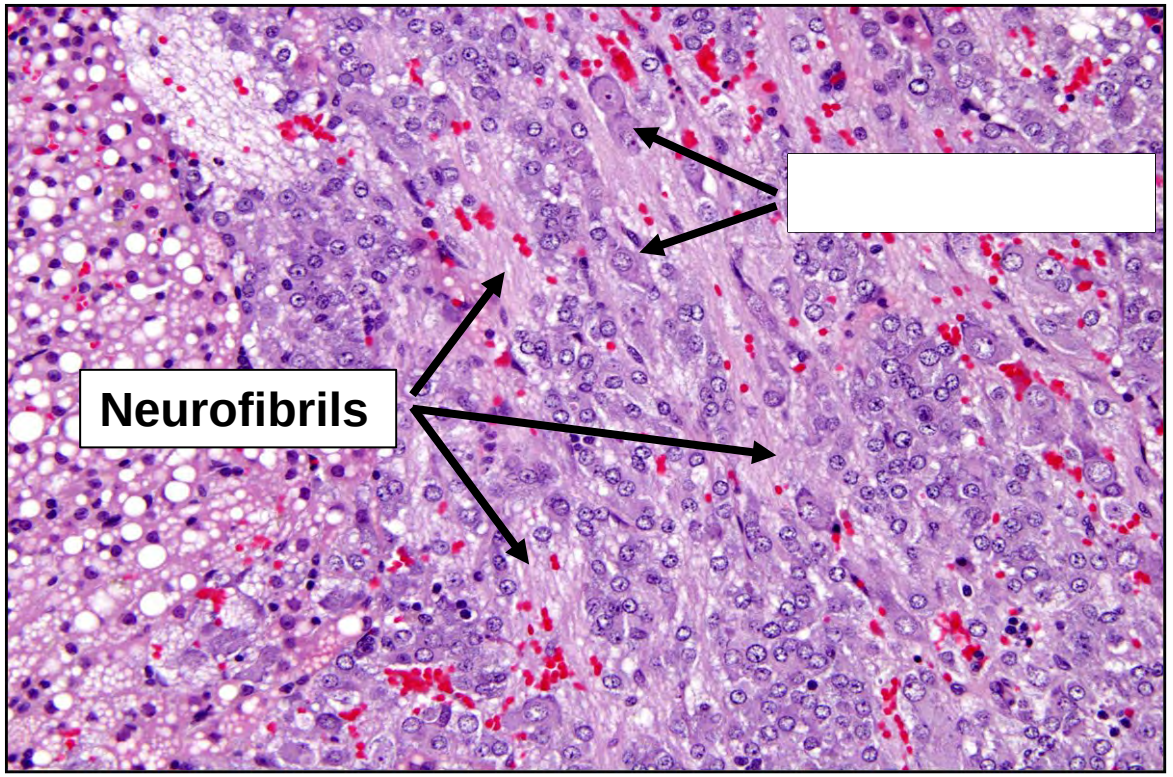
Adrenal Gland – Pheochromocytoma, Complex, Malignant

- Malignant tumor derived from adrenal medullary (chromaffin) cells
- Cell types include ganglion cells with eosinophilic nerve fibers (neurofibrils) and malignant chromaffin cells that may be intermingled or form distinct areas
  - The term “complex” is applied when the chromaffin component is less than 80% of the mass and ganglion cells and nerve fibers (neurofibrils) are present
- Diagnostic features
  - Invasion from the medullary neoplasm into the cortex or through the adrenal capsule or metastasis to distant sites
  - Neoplastic chromaffin cells are usually small with scant basophilic cytoplasm and are arranged in nests, rows and cords surrounding numerous, often distended, blood vessels
  - Cellular atypia may be present, and mitotic figures may be numerous
- Adrenal medullary cells have a positive chromaffin reaction using histochemical stains (e.g., Cherukian-Schenck stain) and are immunohistochemically positive for tyrosine hydroxylase, chromogranin A, synaptophysin or neuron-specific enolase
- Observed in mice and rats
- Differential diagnoses: Pheochromocytoma, complex, benign; Pheochromocytoma, malignant; Neuroblastoma, malignant; Carcinoma, cortex

Adrenal Gland – Pheochromocytoma, Complex, Malignant



A malignant complex pheochromocytoma from a rat expanding the medulla and invading into the cortex (arrows).



The neoplasm contains a mixture of neoplastic ganglion cells, eosinophilic neurofibrils, and chromaffin cells.

Summary

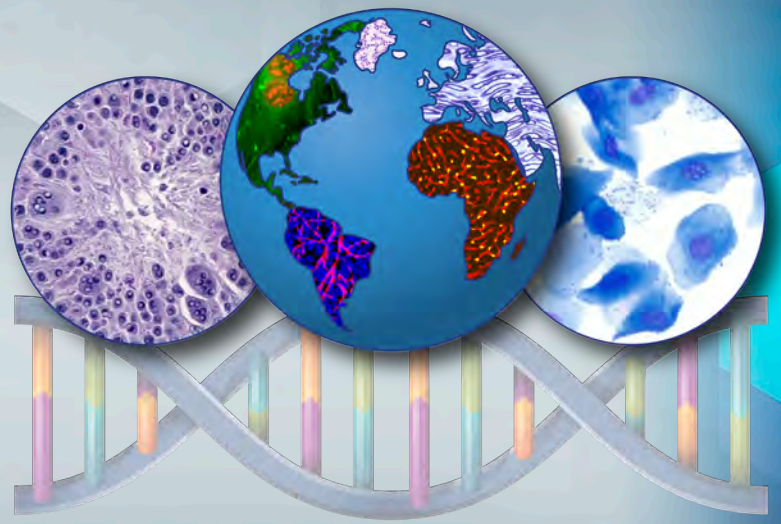
- Proliferative lesions of the adrenal gland are a relatively common finding in rodent toxicology studies and can be age and strain related
- Hyperplastic lesions of the adrenal cortex and medulla likely represent a continuum with neoplasia and differentiating between the two processes can be challenging
- The use of standard diagnostic features can aid in distinguishing proliferative lesions of the adrenal gland

References

- goRENI (https://www.goreni.org/gr3_index.php)
- INHAND- Nonproliferative and Proliferative Lesions of the Rat and Mouse Endocrine System (https://www.toxpath.org/docs/WED_Endocrine.pdf)
- Nonneoplastic Lesion Atlas (https://ntp.niehs.nih.gov/atlas/nnl)
- Nyska A, Maronpot RR. 1990. Adrenal gland. In: Pathology of the Mouse: Reference and Atlas (Maronpot RR, Boorman GA, Gaul BW, eds). Cache River Press, Vienna, IL, 509-536.
- Suttie AW, Sutcliffe C. 2018. Adrenal Gland. In Suttie AW (ed.) *Boorman's Pathology of the Rat,* pp. 649 – 666. Academic Press. Cambridge, MA.

National Institute of Environmental Health Sciences
Division of Translational Toxicology

- Author
  - Marlene Orandle, DVM, PhD – Inotiv-RTP
- Reviewers
  - Beth Lubeck, PhD, MBA – Division of Translational Toxicology (DTT), NIEHS
  - Cynthia Willson, MS, PhD, DVM, DACVP, DABT – Inotiv-RTP
  - Tom Steinbach, DVM, DACVP, DABT – EPL, Inc



Division of Translational Toxicology
Global Toxicologic Pathology Training Program