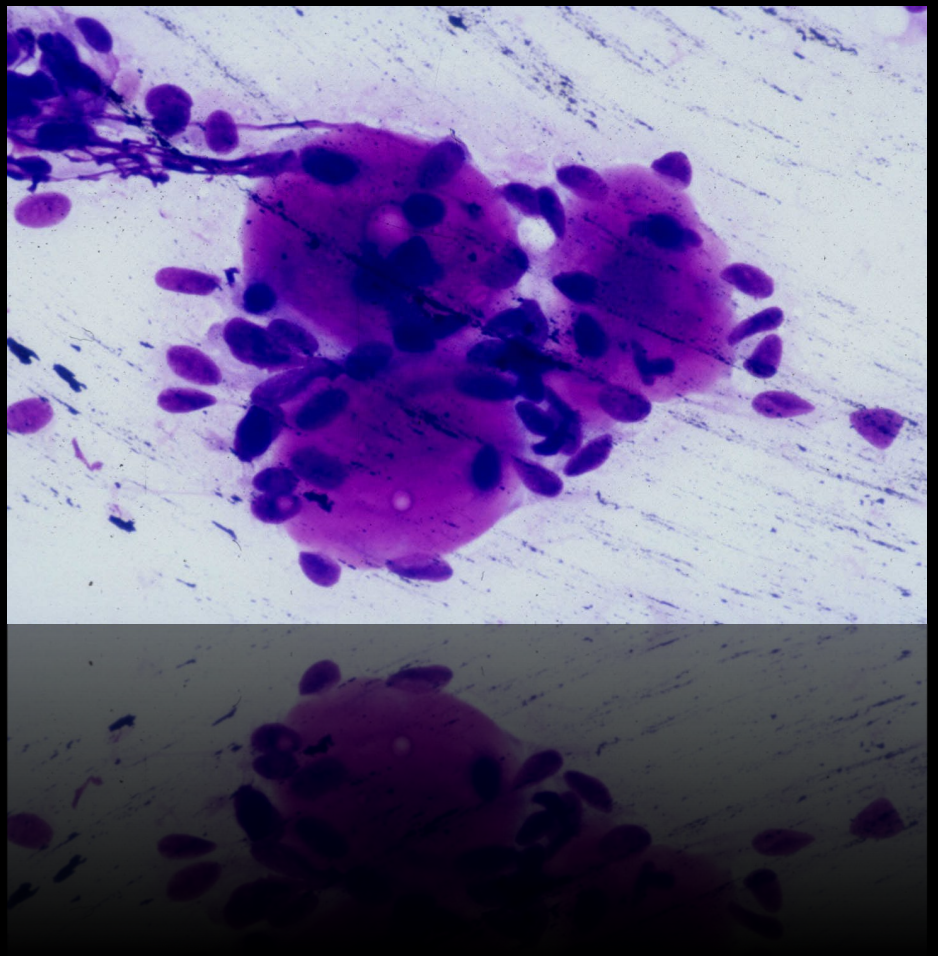
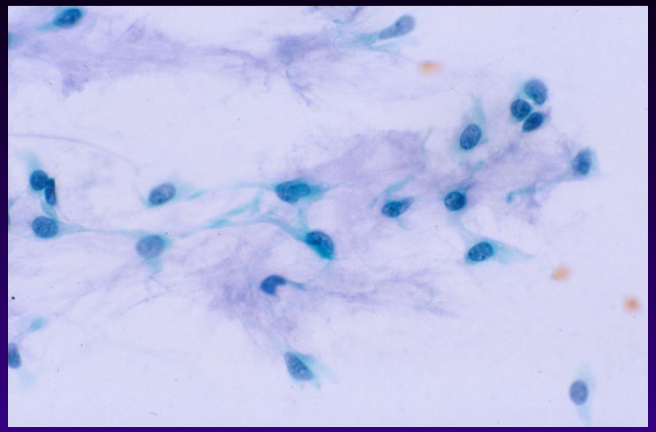
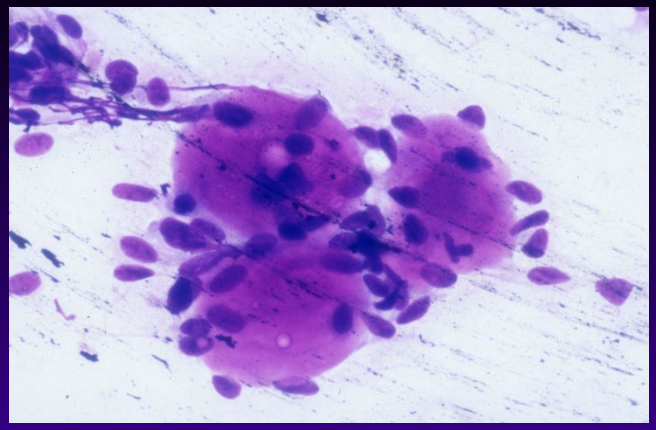
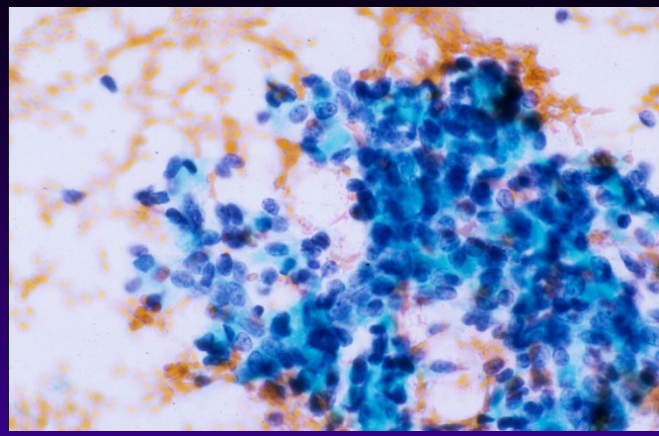
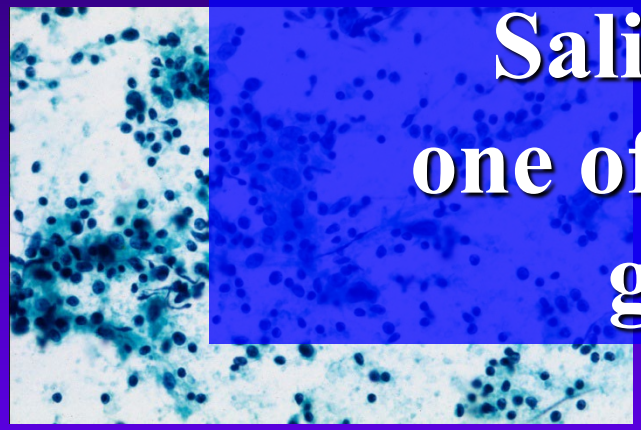
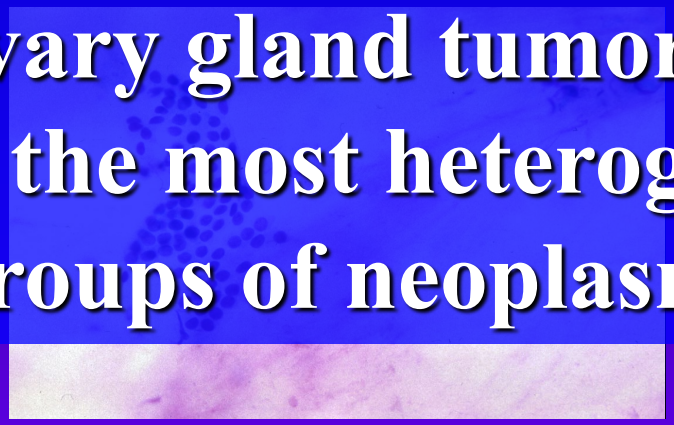
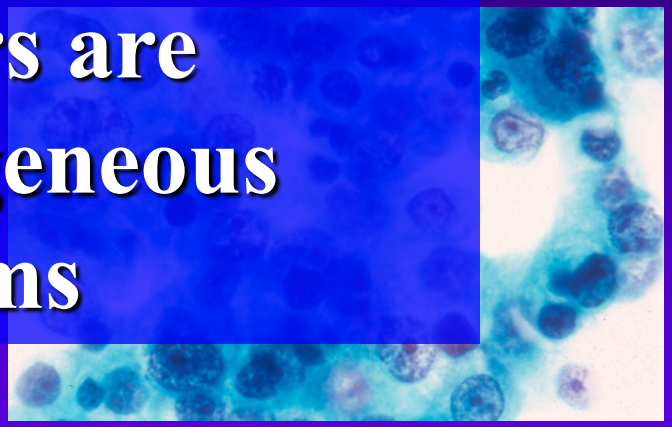
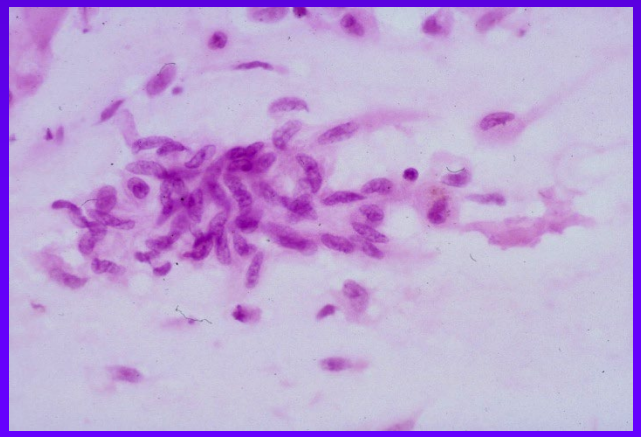
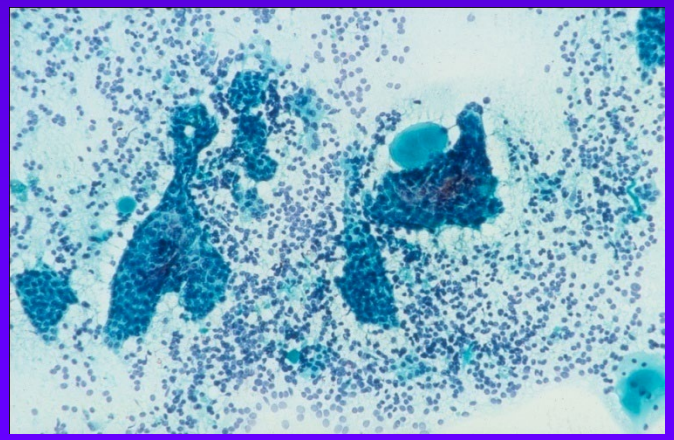
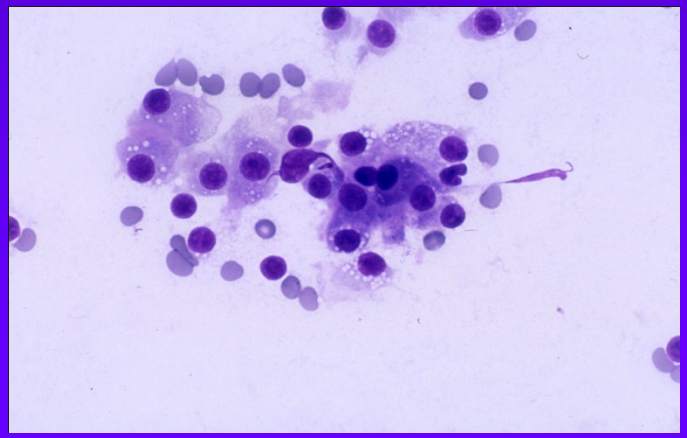
William C. Faquin, MD, PhD
Professor of Pathology
Harvard Medical School
Massachusetts General Hospital

Director of Head and Neck Pathology
Massachusetts Eye and Ear

@BFAQUIN



Essentials in Salivary Gland Cytology

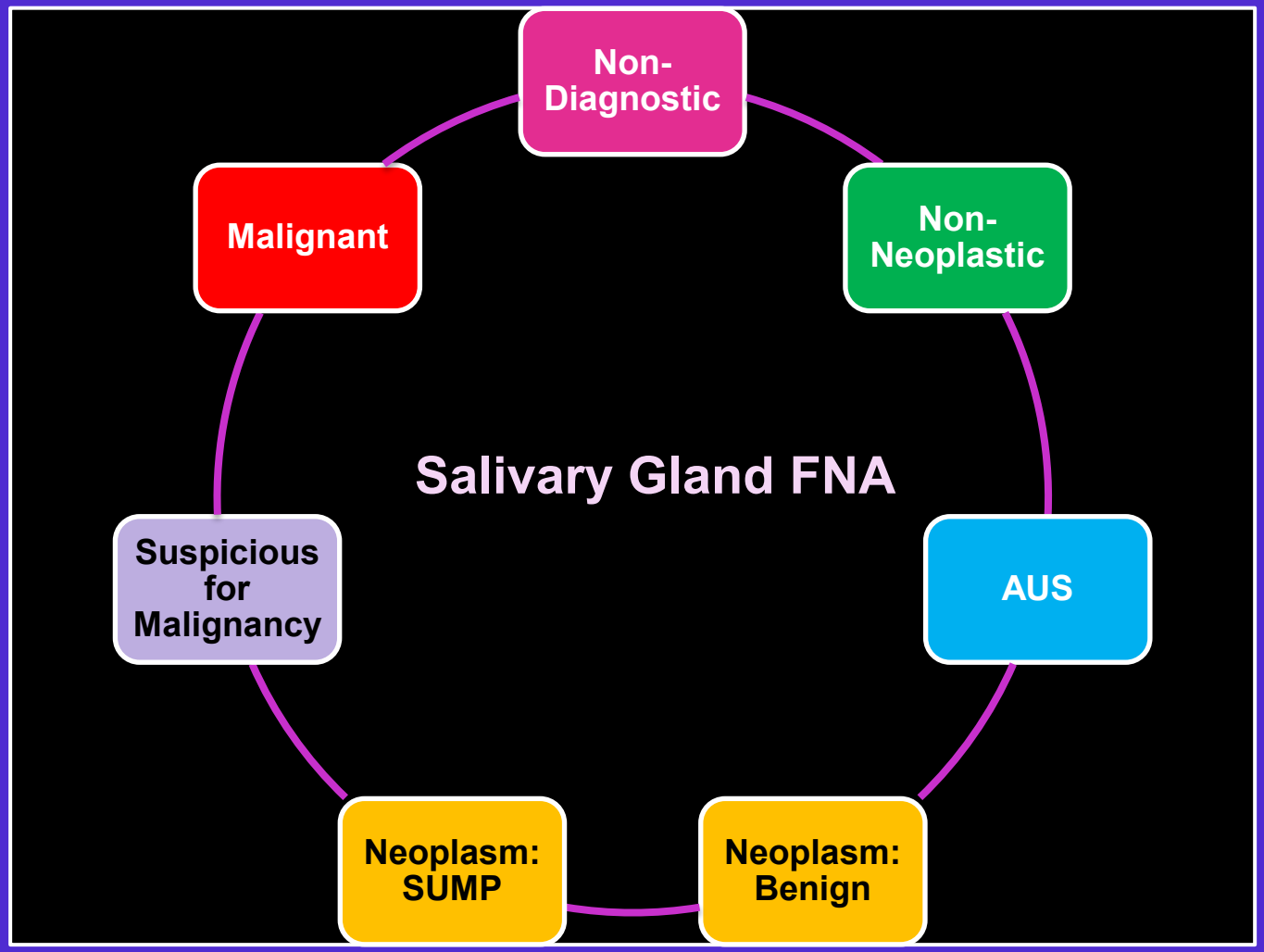
Salivary gland tumors are one of the most heterogeneous groups of neoplasms

This makes salivary gland cytology especially challenging due to the extensive overlap between many benign and malignant tumors.

What is the Milan System for Reporting Salivary Gland Cytopathology?

The Milan System for Reporting Salivary Gland Cytopathology:

Diagnostic Categories



Are there molecular signatures for most salivary gland tumors?

Increasing Availability of Molecular Markers For Salivary Gland Tumors

- Secretory carcinoma:
  - ETV6-NTRK3; t(12:15)
- Pleomorphic adenoma & Ca ex PA:
  - PLAG1; t(3;8)
  - HMGA2 rearrangement
- Clear cell carcinoma:
  - EWSR1-ATF1; t(12:22)
- Mucoepidermoid carcinoma:
  - MECT1/MAML2; t(11;19)

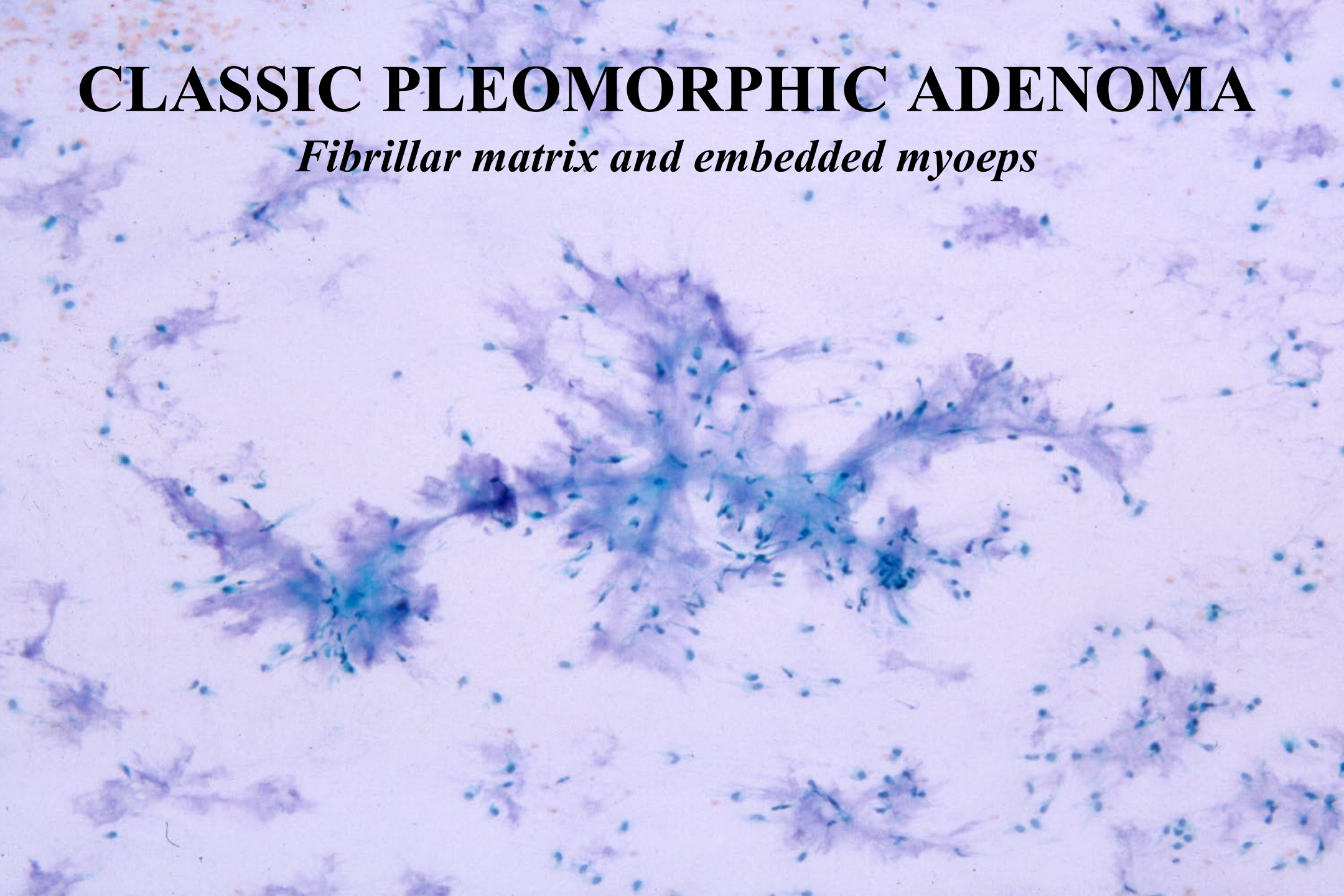
FISH is good for a specific entity
Multiplex PCR is best for a DDX

- Adenoid cystic carcinoma:
  - MYB-NFIB; t(6:9)
- Basal cell adenoma:
  - CTNNB1 mutation
- Acinic cell carcinoma
  - NR4A3; t(4:9)
- Epithelial-myoepithelial carcinoma
  - RAS mutation
- Intraductal carcinoma
  - NCOA4-RET

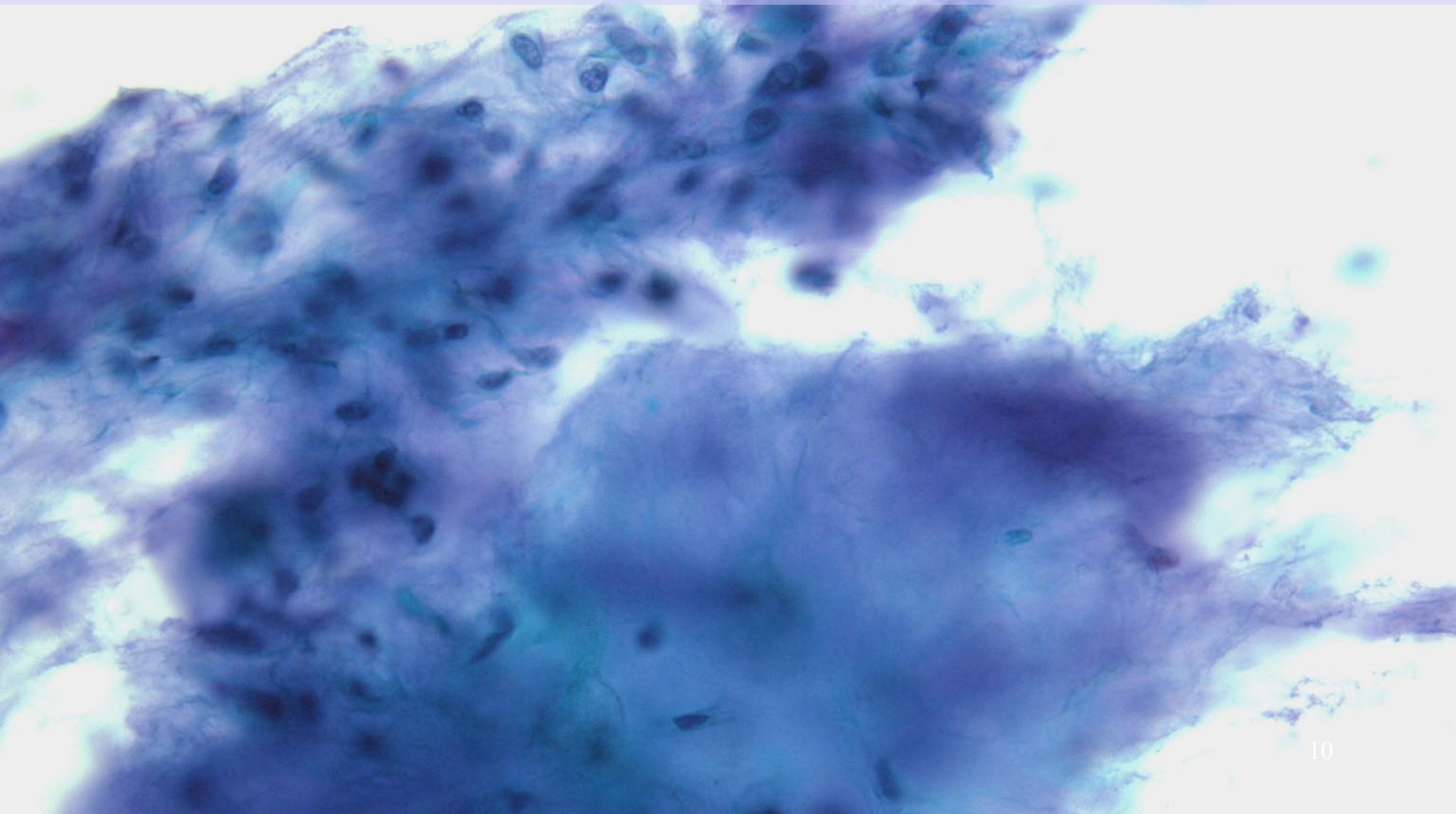
FNA of Matrix-Producing Salivary Gland Tumors

CLASSIC PLEOMORPHIC ADENOMA

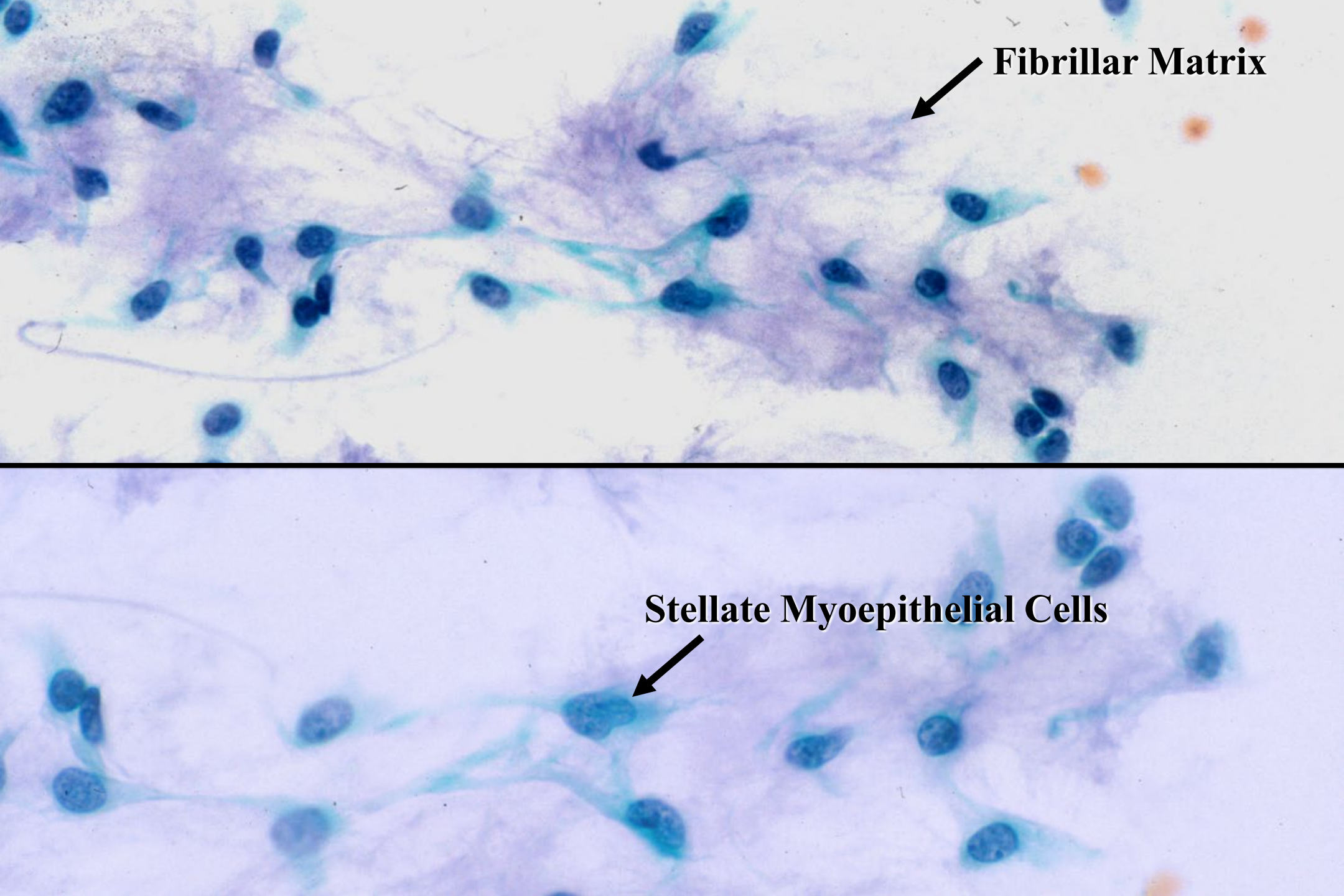
Fibrillar matrix and embedded myoeps



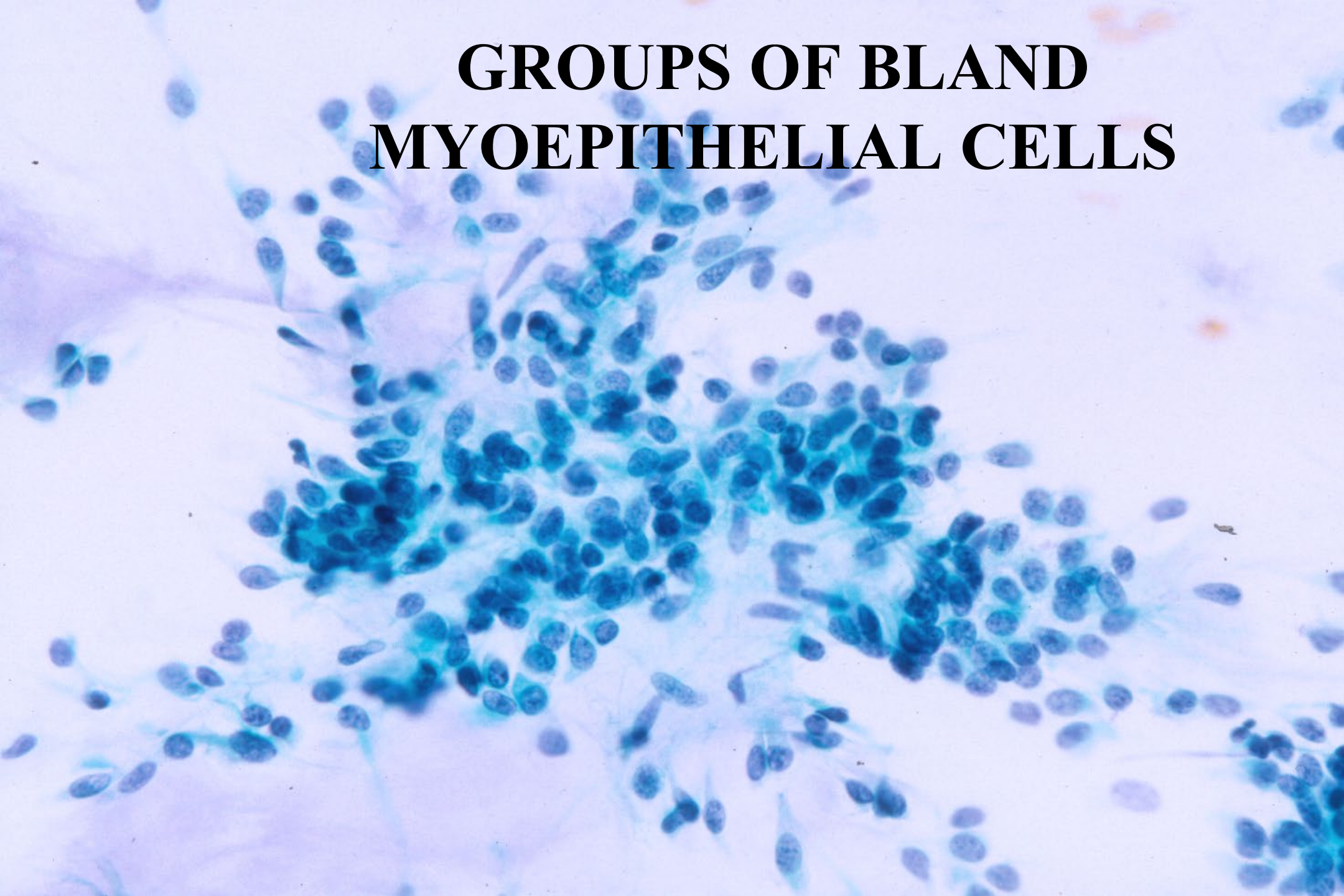
Pleomorphic Adenoma Matrix



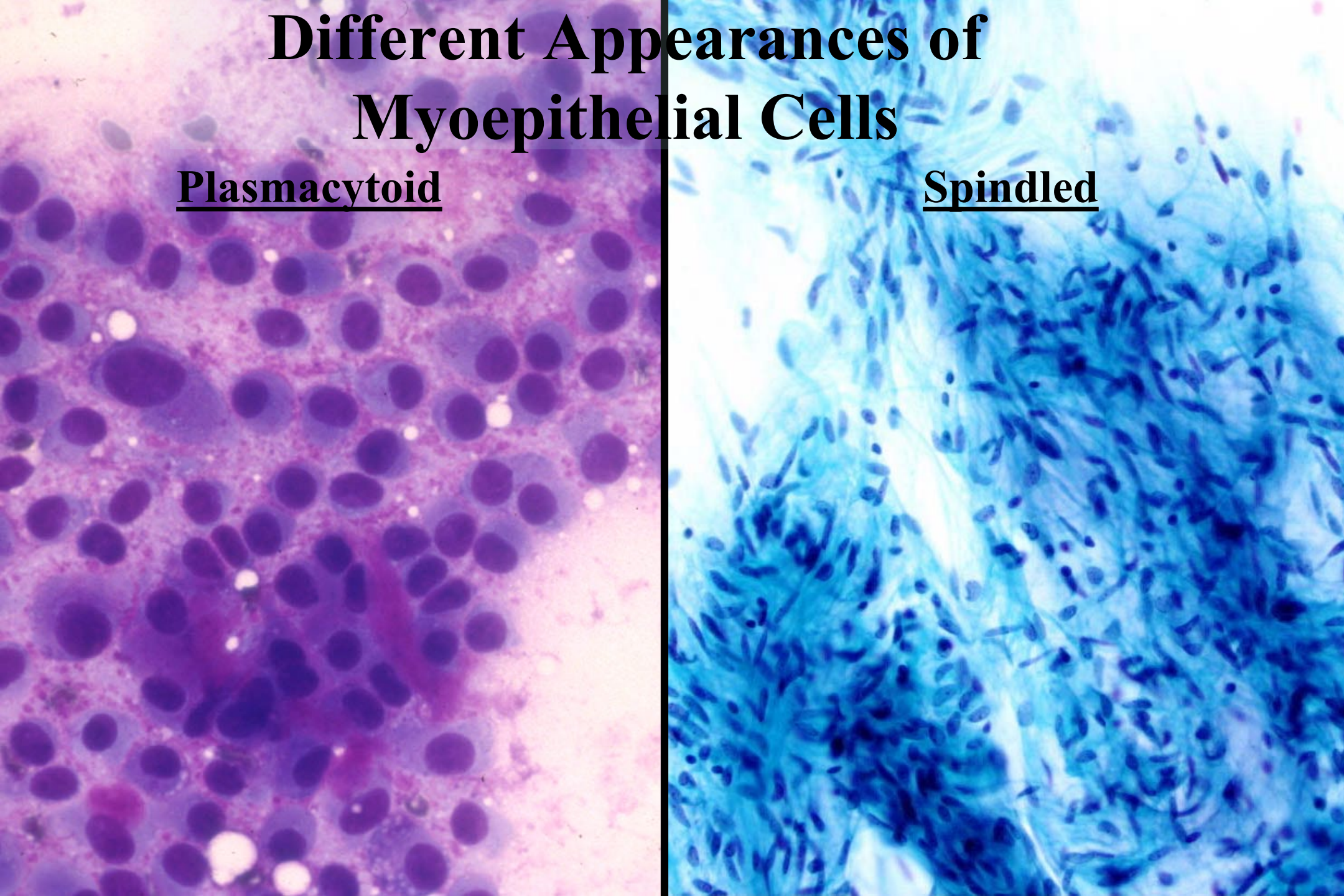
Metachromatic Matrix in Air-Dried Preparations


Fibrillar Matrix
Stellate Myoepithelial Cells

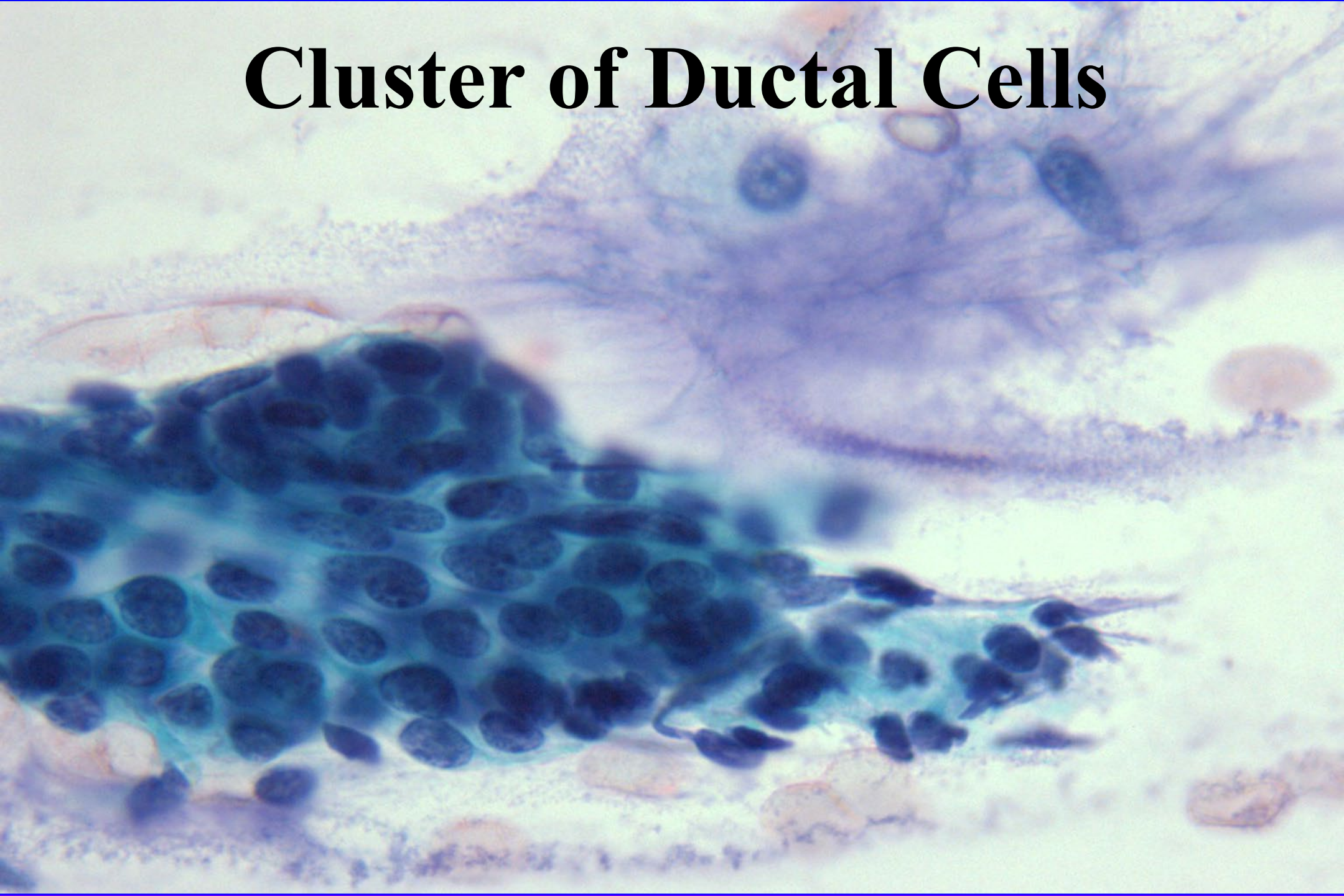
GROUPS OF BLAND MYOEPITHELIAL CELLS



Different Appearances of Myoepithelial Cells



Cluster of Ductal Cells



KEY POINTS

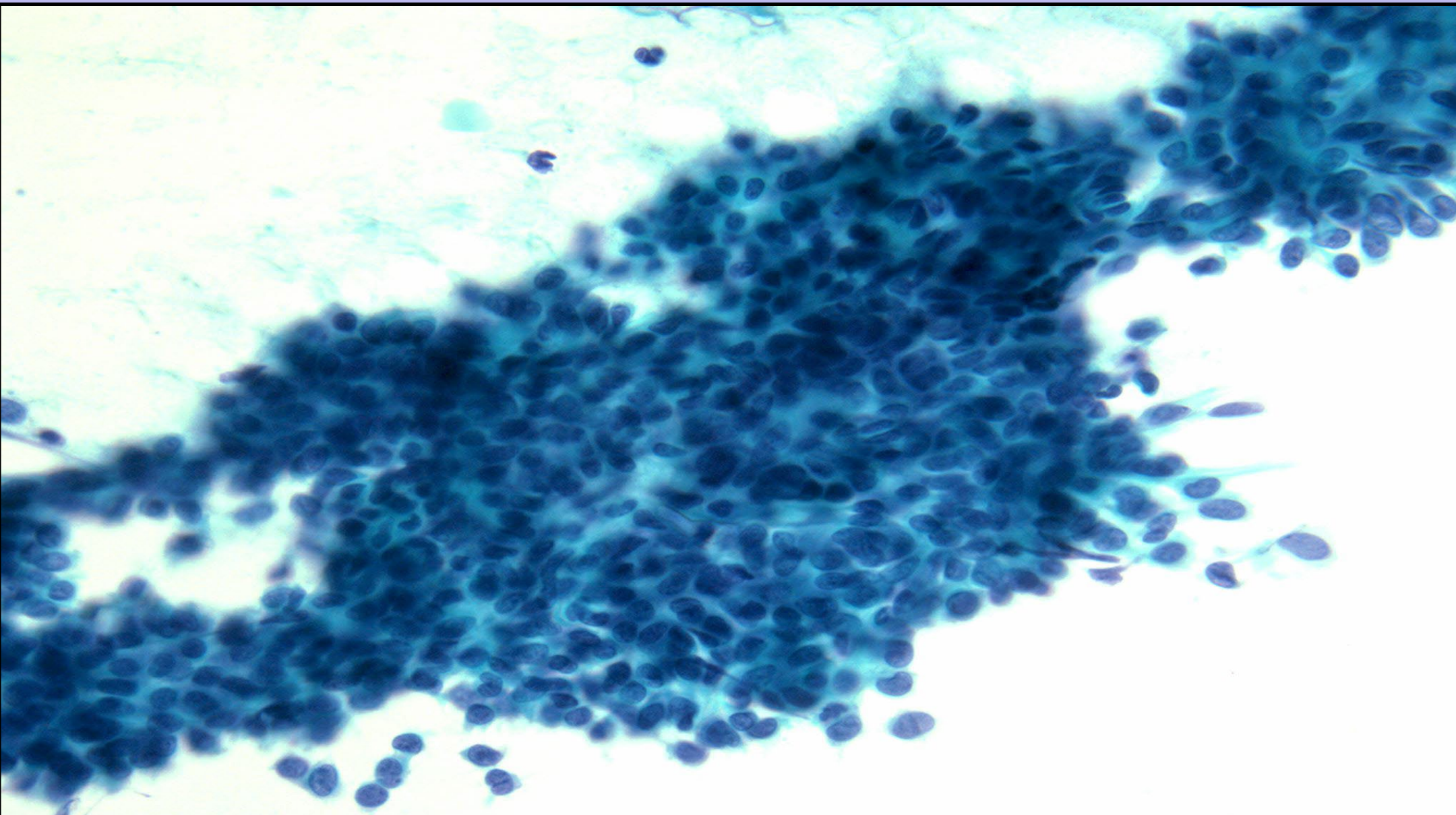
- Pleomorphic Adenoma Cytology
  - Fibrillar matrix with irregular edges
  - Embedded myoepithelial cells
  - Occasional groups of ductal cells
  - Most are easily classified as **BENIGN**
  - **PLAG1 gene fusions**

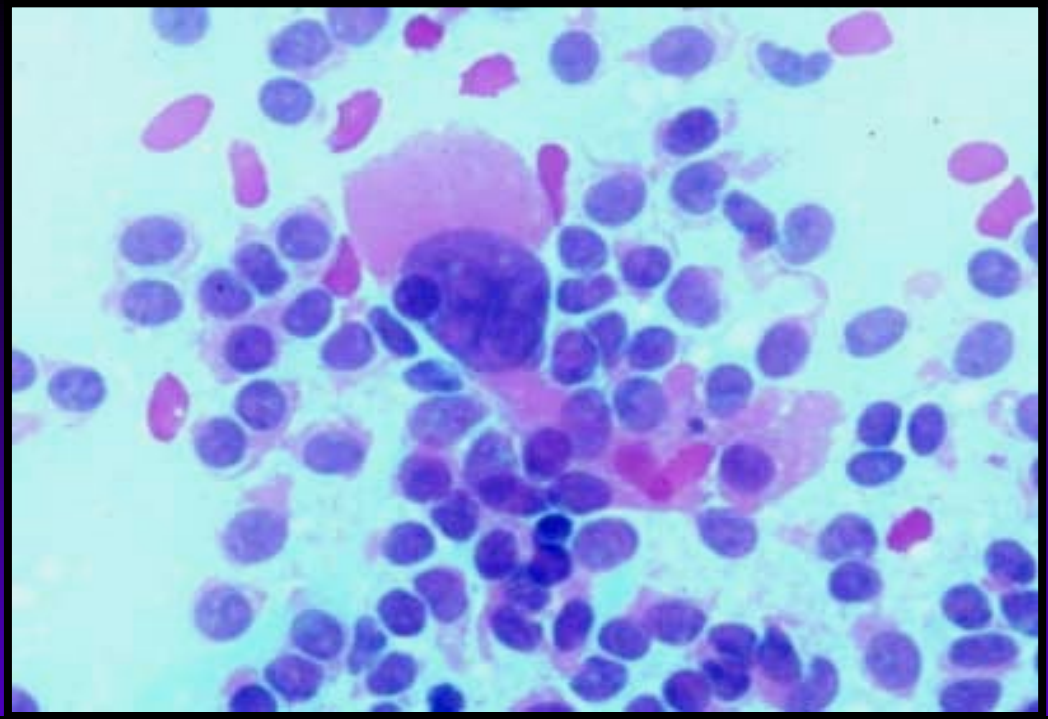
VARIATIONS OF PLEOMORPHIC ADENOMA

- Cellular PA with sparse matrix
- Focal adenoid cystic–like areas
- Cytologic atypia
- Metaplasia
  - Squamous
  - Mucinous
  - Sebaceous
  - Oncocytic

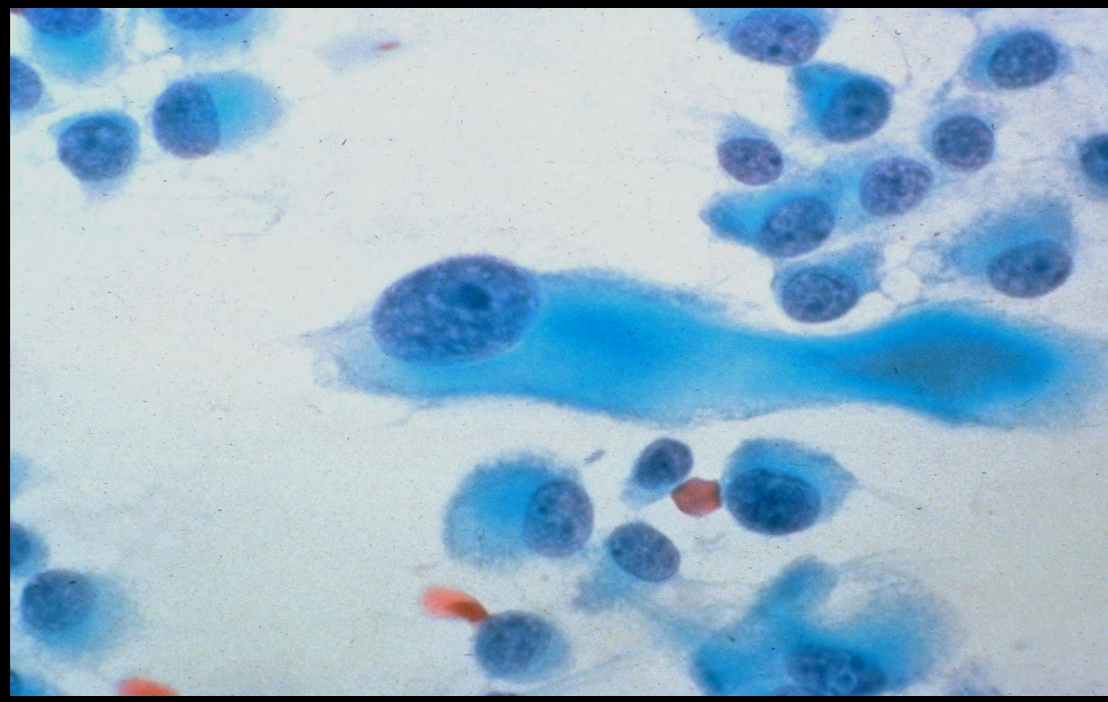
Cellular Pleomorphic Adenoma

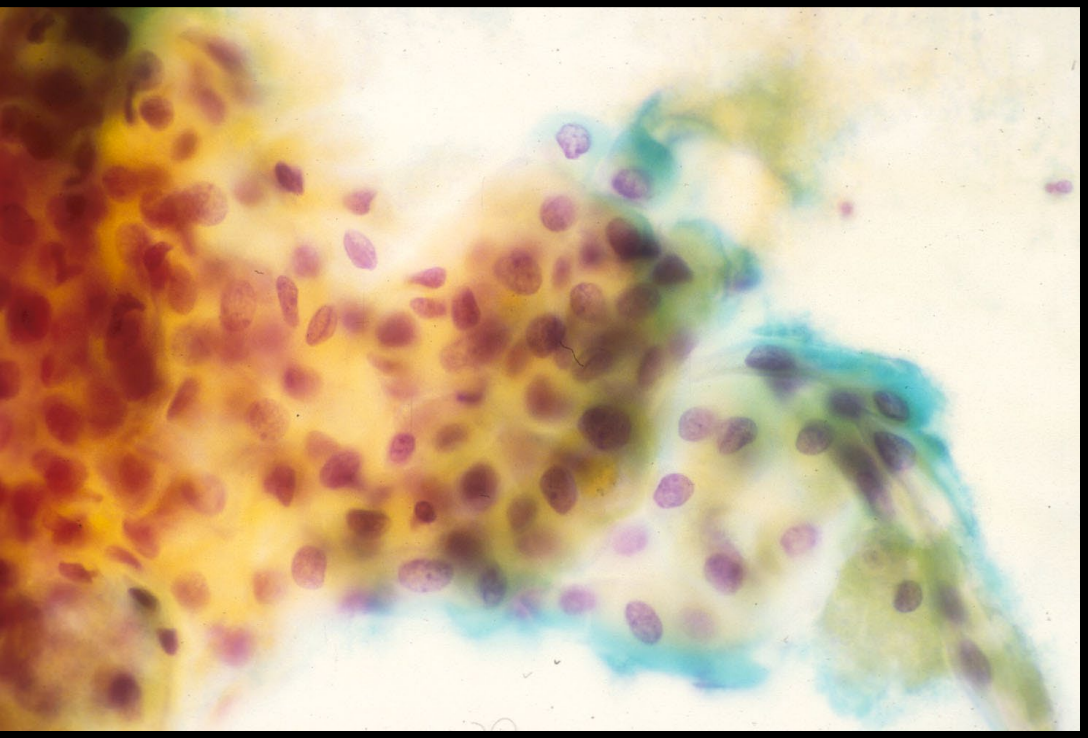
Scant matrix but myoepithelial predominant

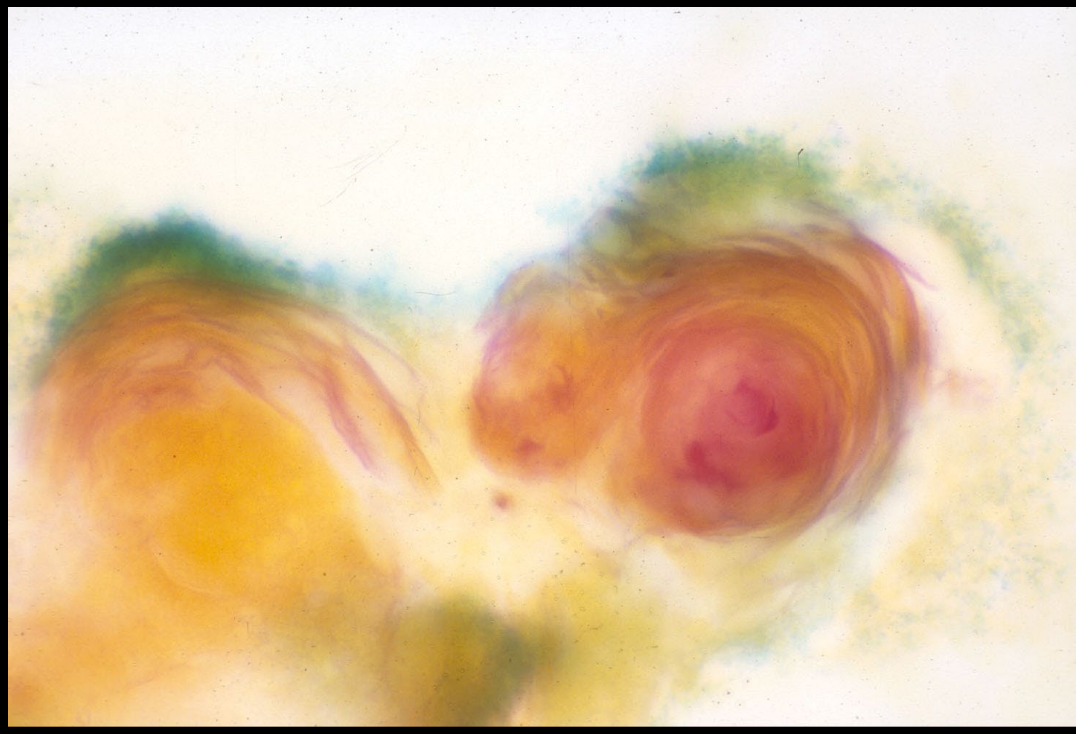


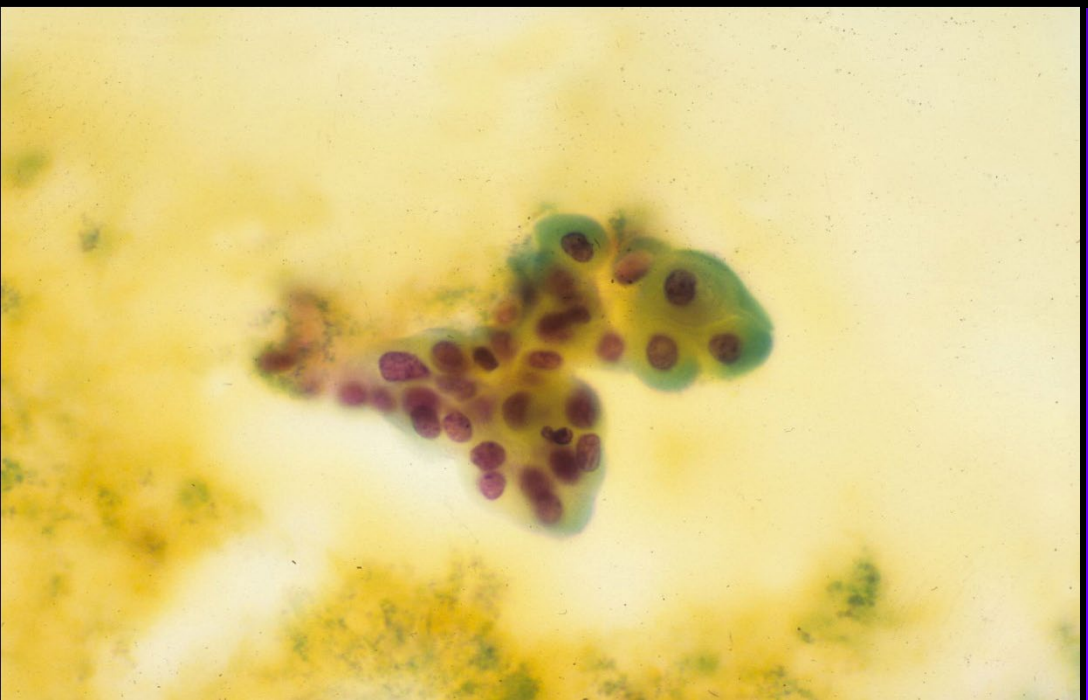


Focal Cytologic Atypia is OK !









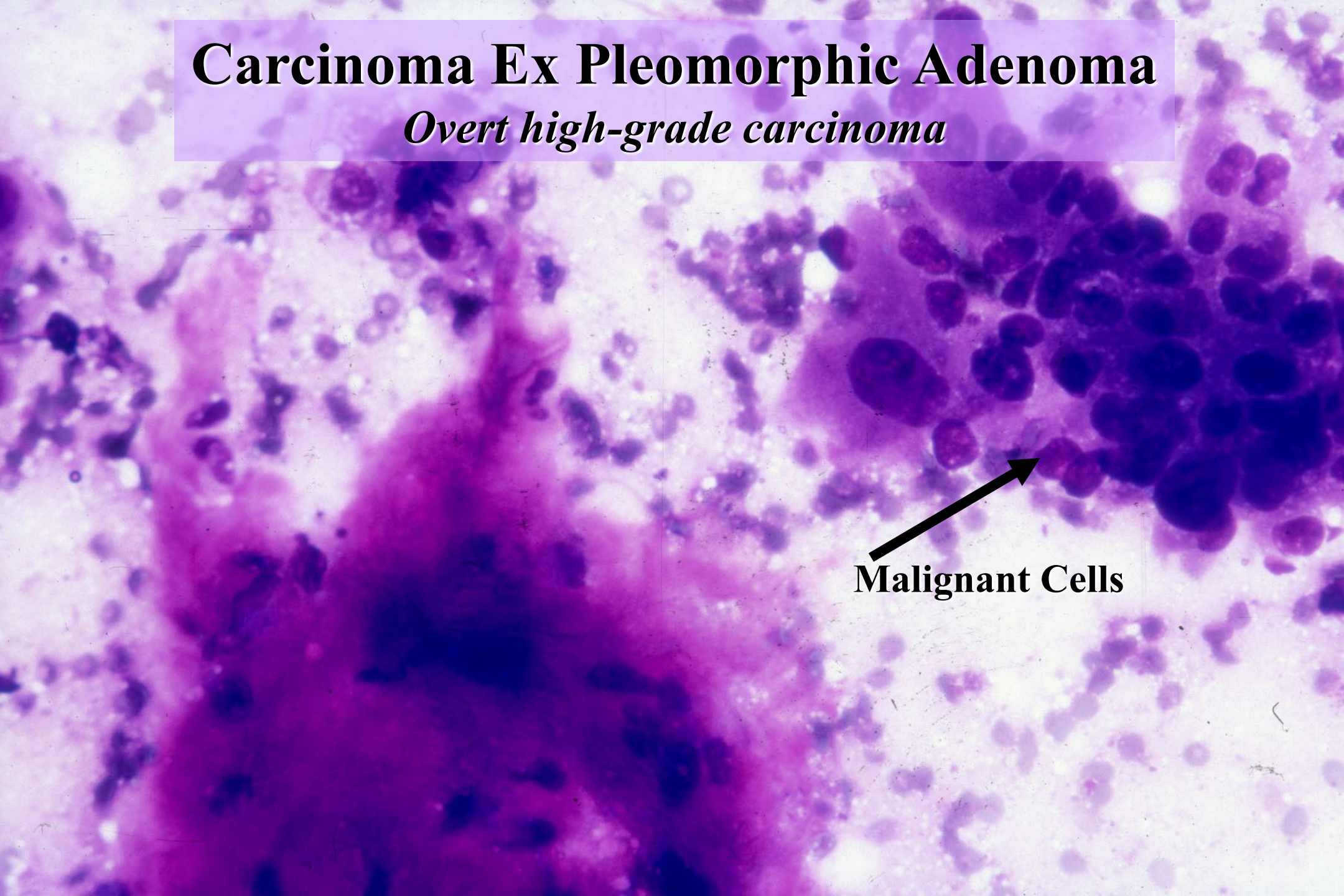
Pitfall:

PA with Squamous Metaplasia

What does carcinoma ex pleomorphic adenoma look like by FNA?

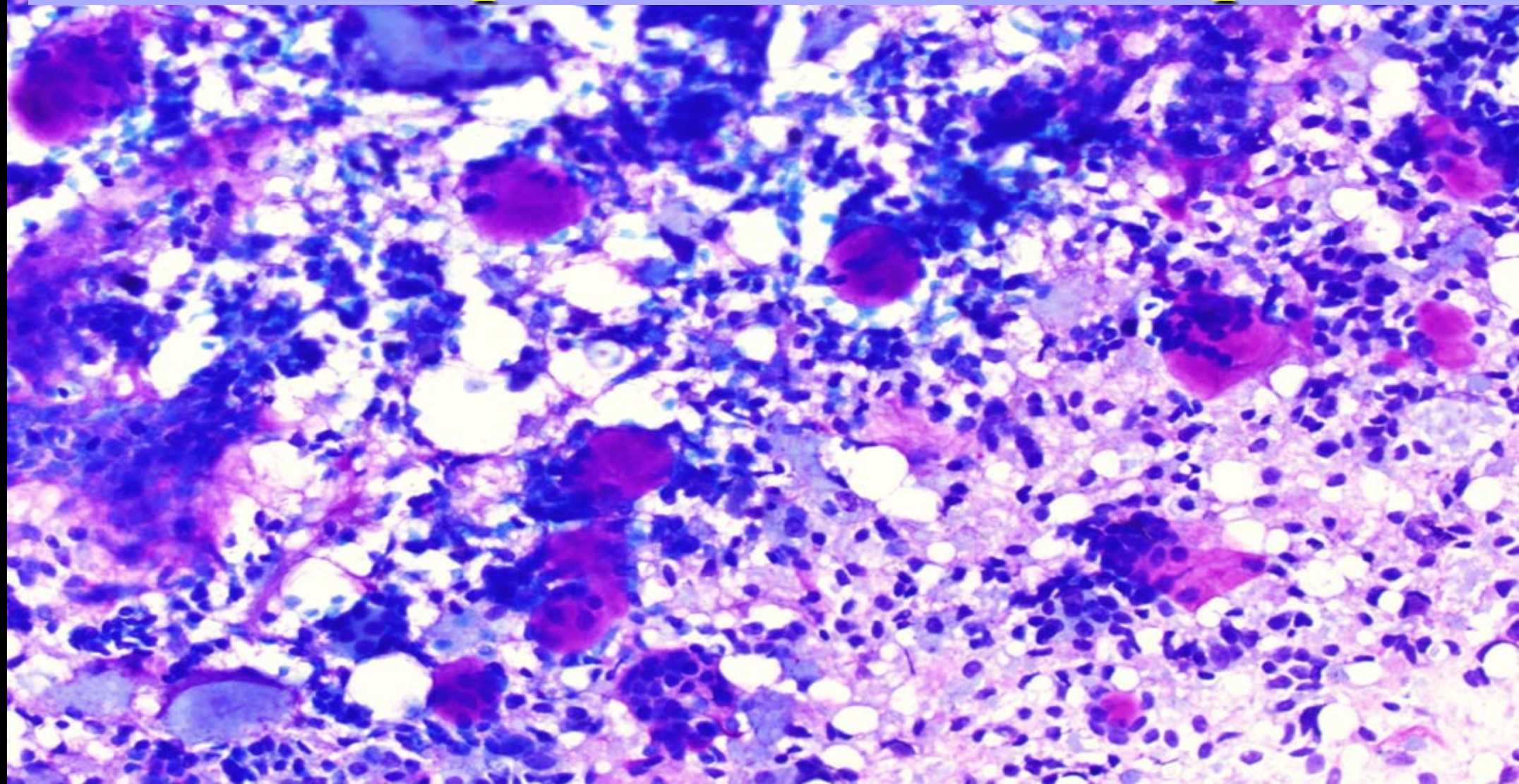
Carcinoma Ex Pleomorphic Adenoma

Overt high-grade carcinoma

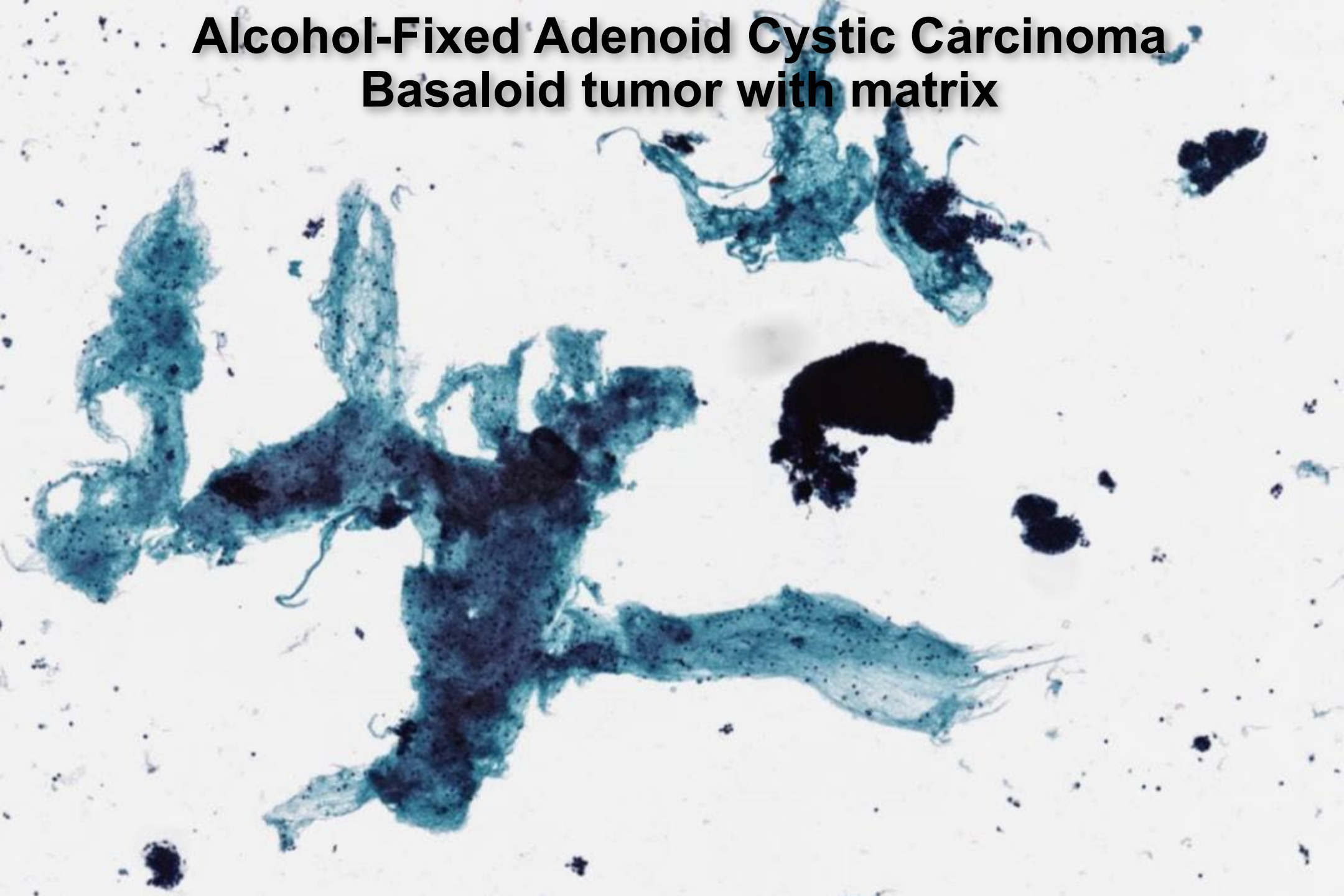


How does adenoid cystic carcinoma compare to pleomorphic adenoma?

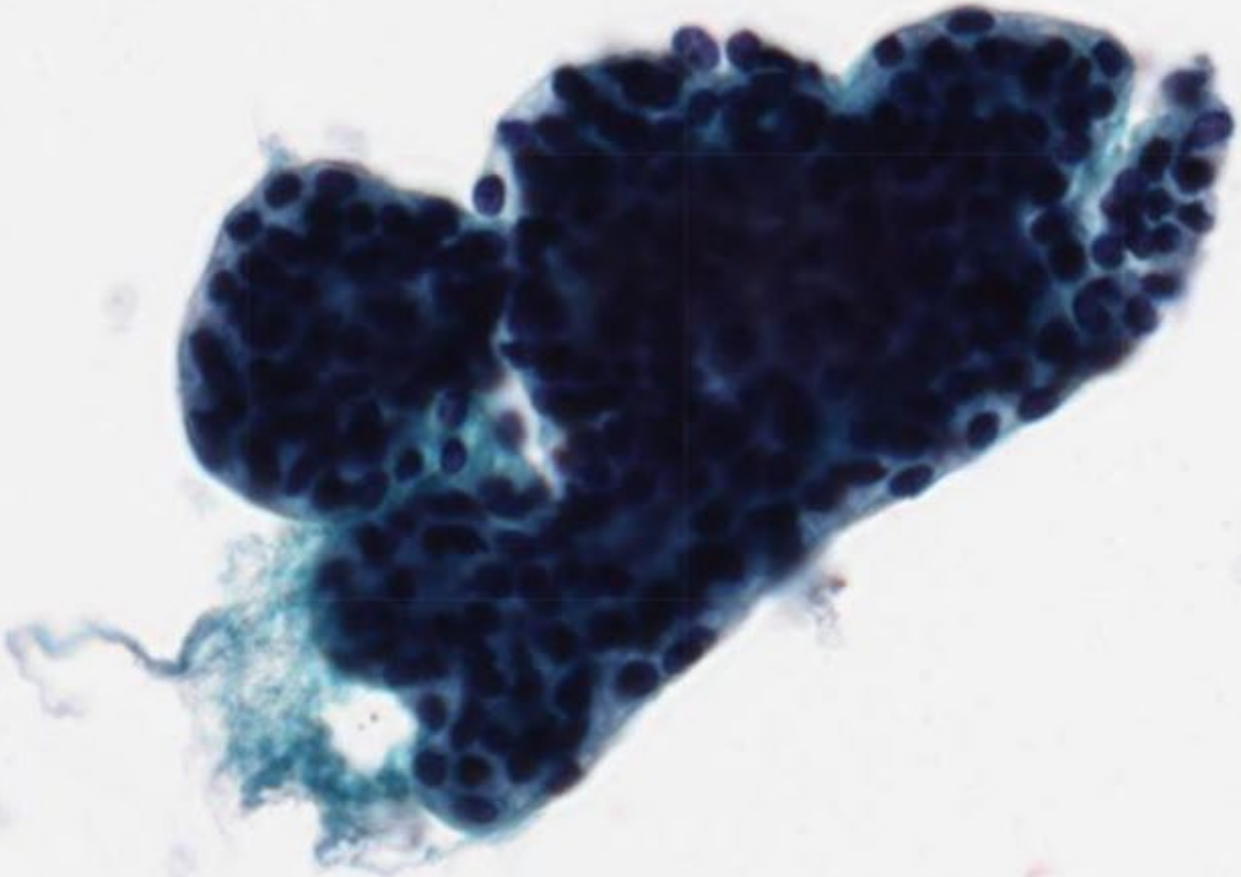
Adenoid Cystic Carcinoma
Patients present with nerve pain!



Alcohol-Fixed Adenoid Cystic Carcinoma
Basaloid tumor with matrix

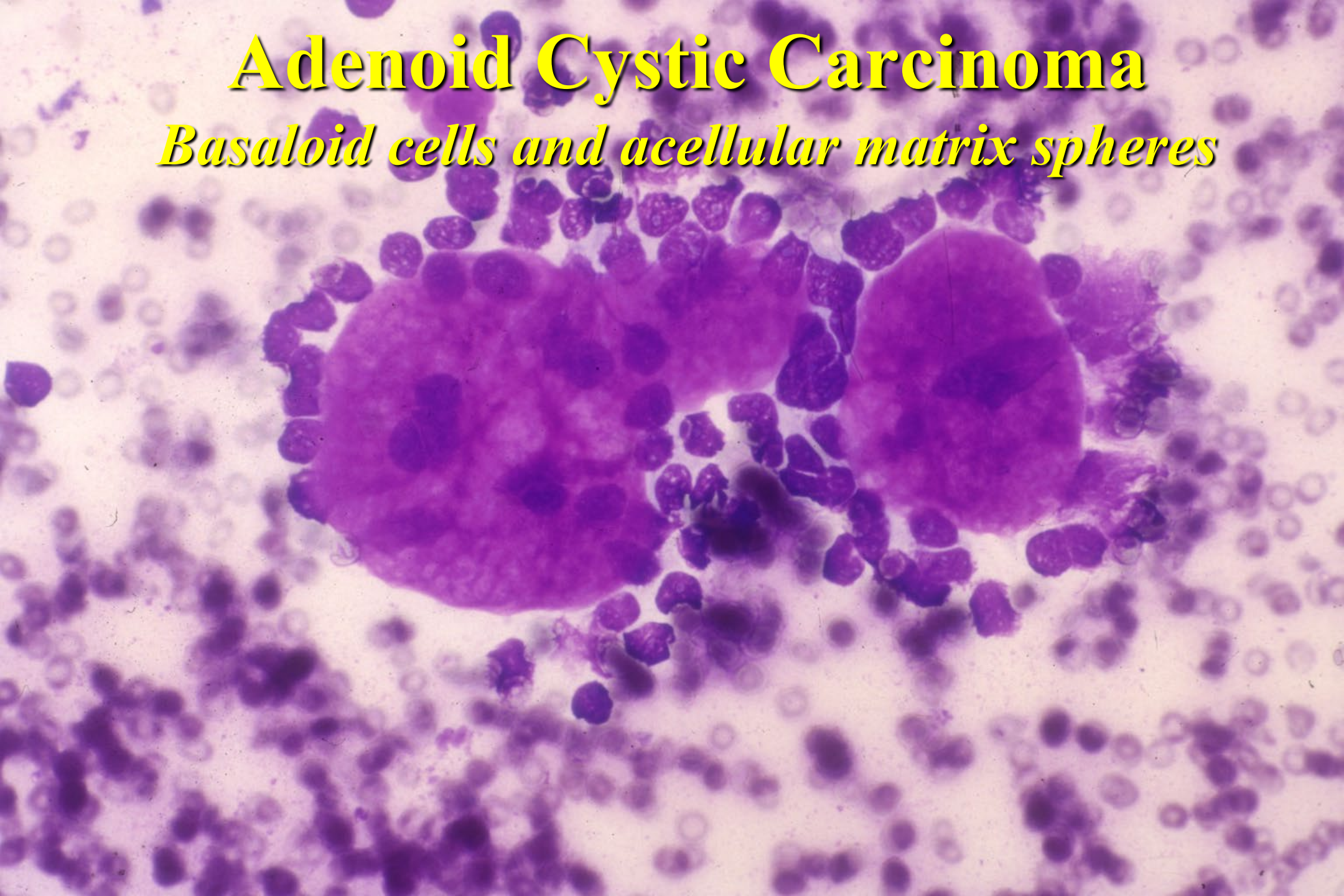


Sharp edges are very worrisome!



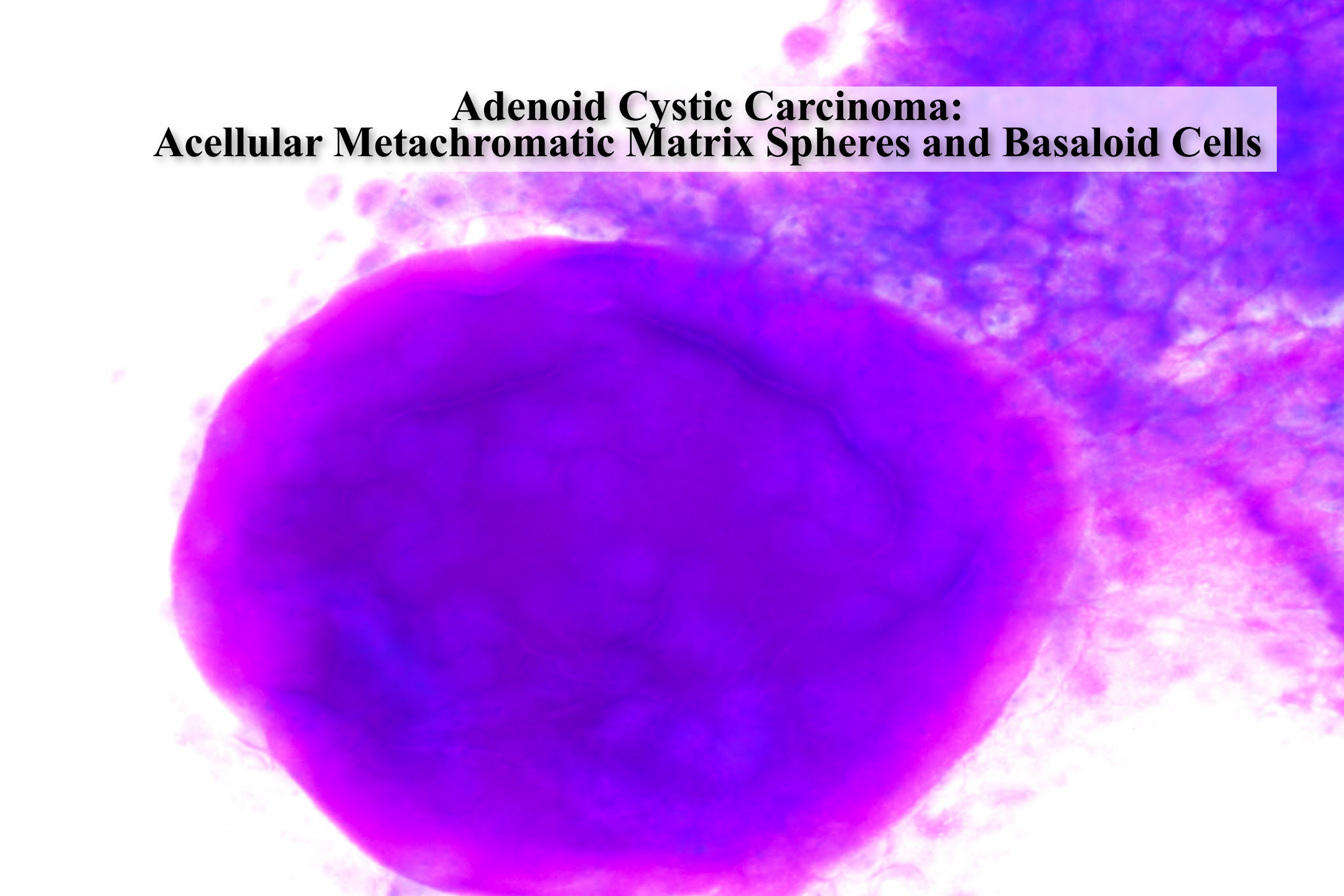
Adenoid Cystic Carcinoma

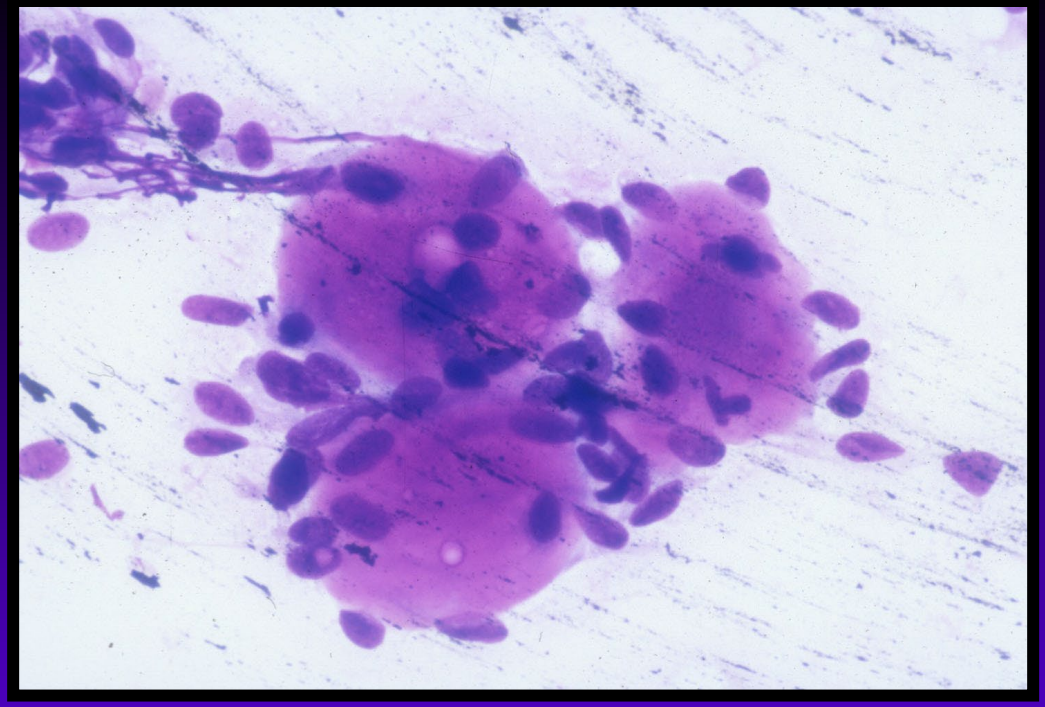
Basaloid cells and acellular matrix spheres

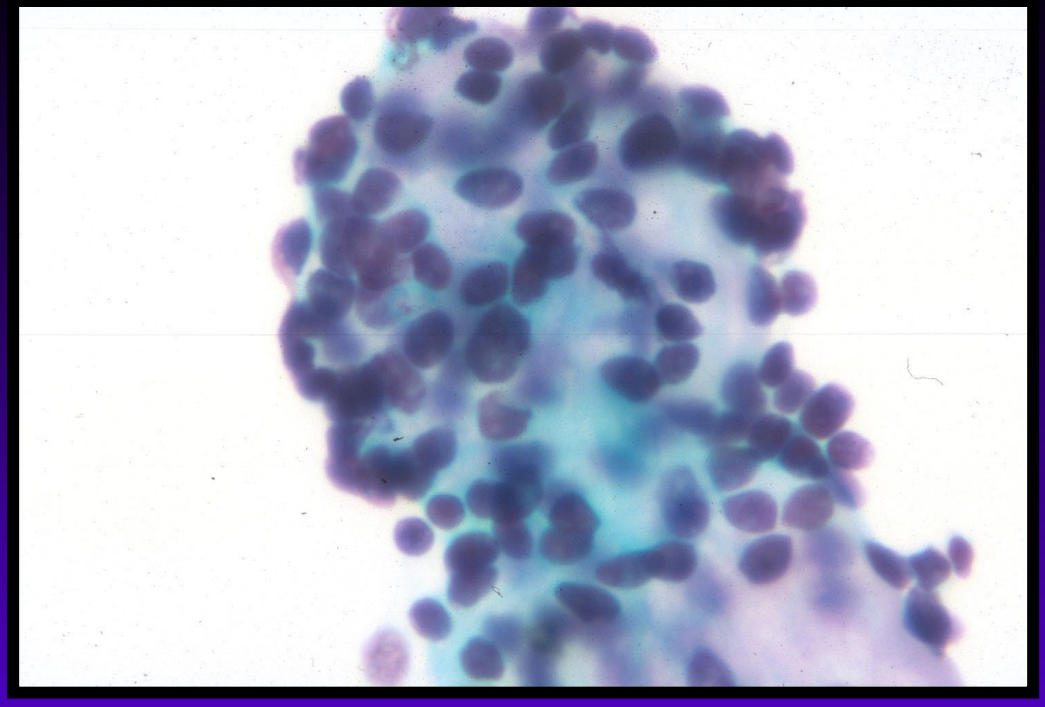


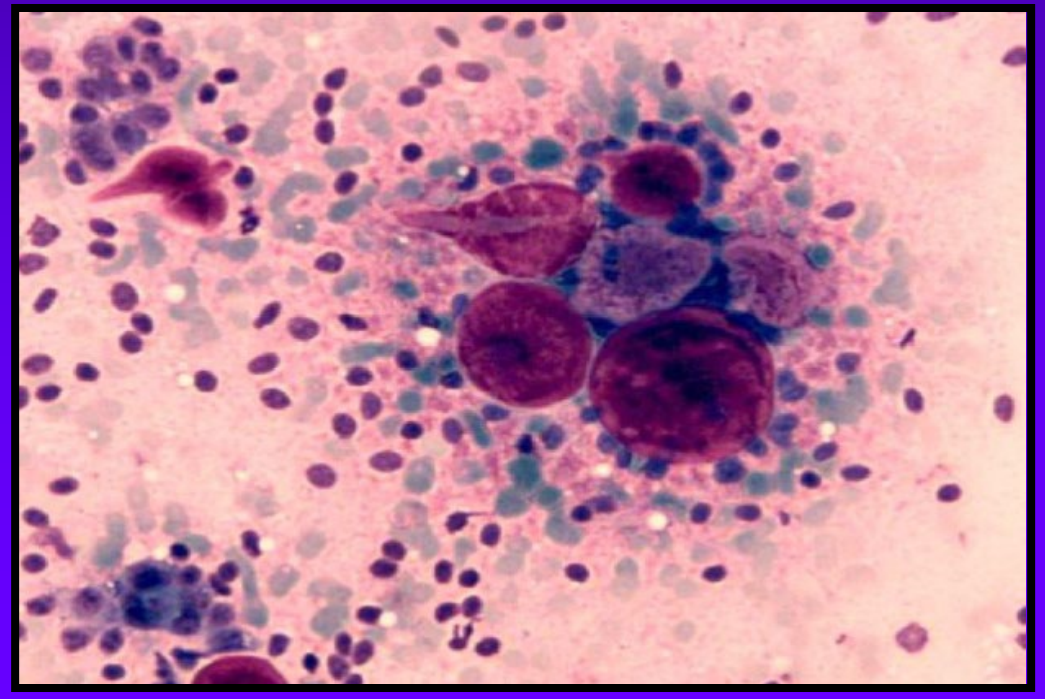

Adenoid Cystic Carcinoma:
Classic Cribriform Pattern

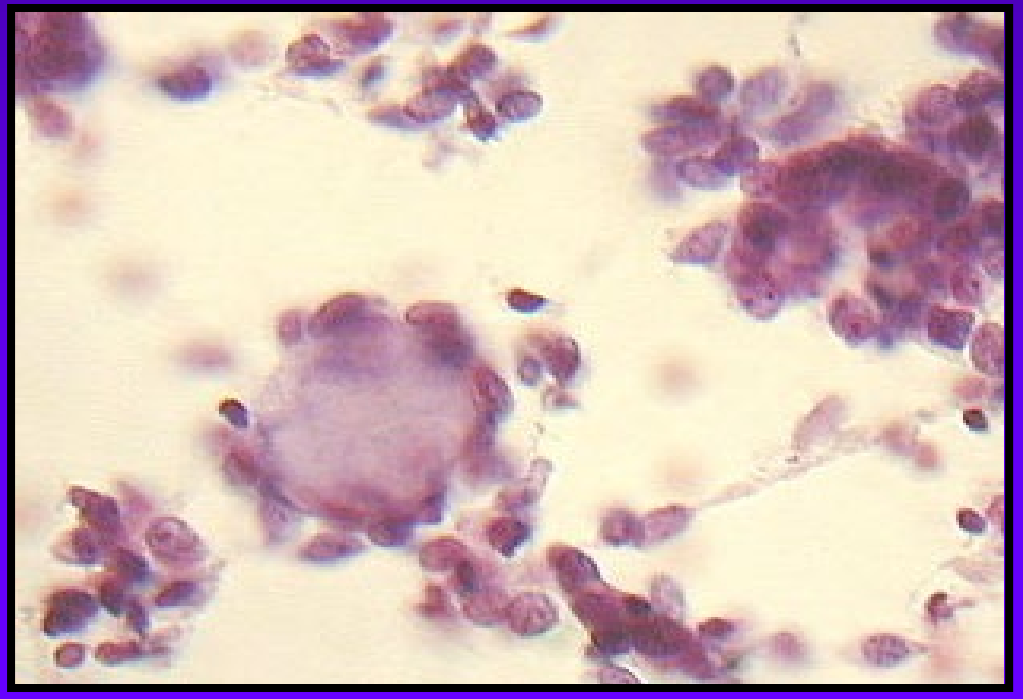
Adenoid Cystic Carcinoma: Acellular Metachromatic Matrix Spheres and Basaloid Cells





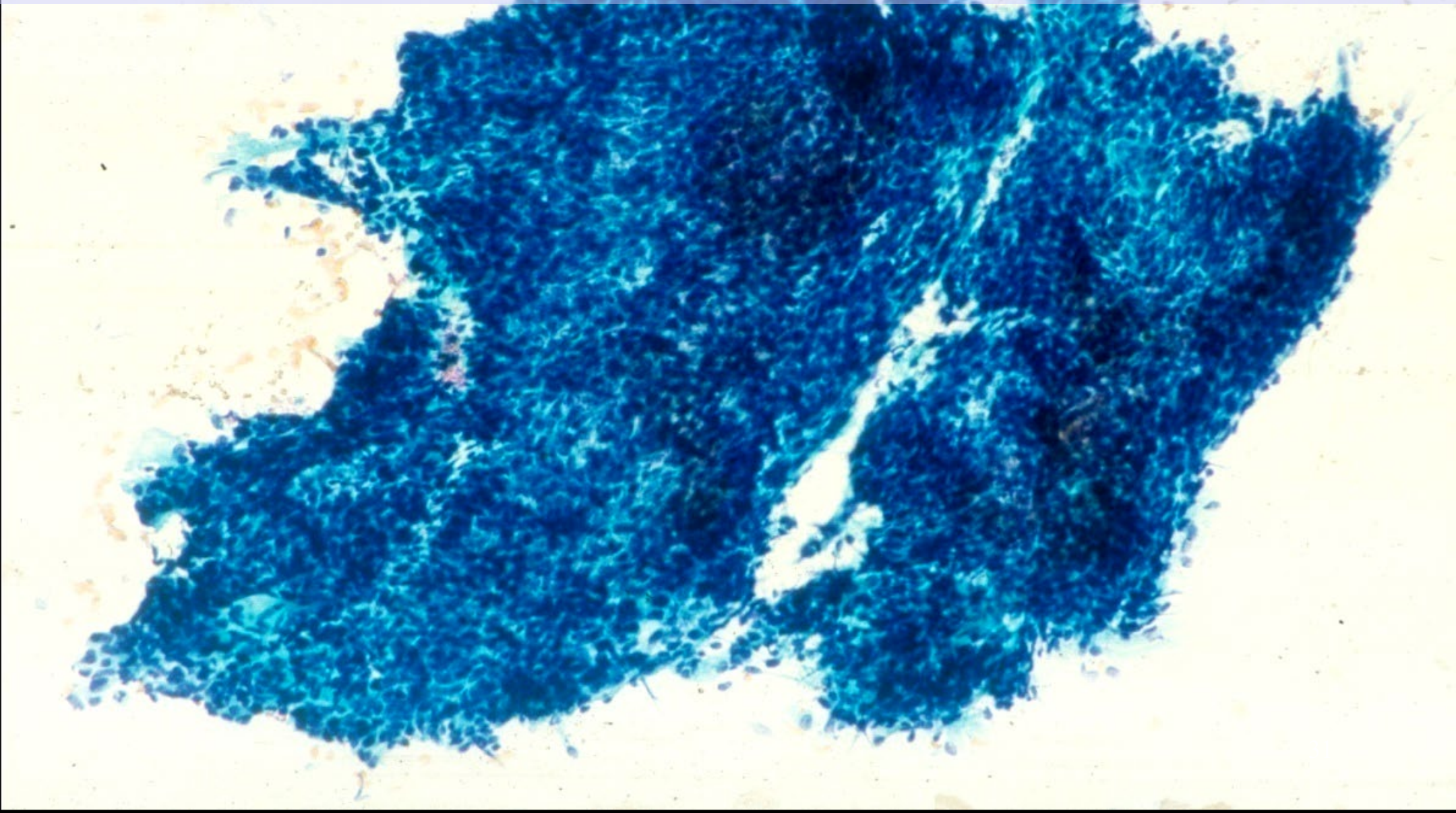


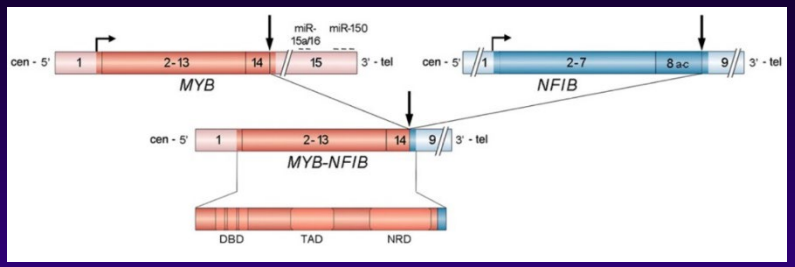




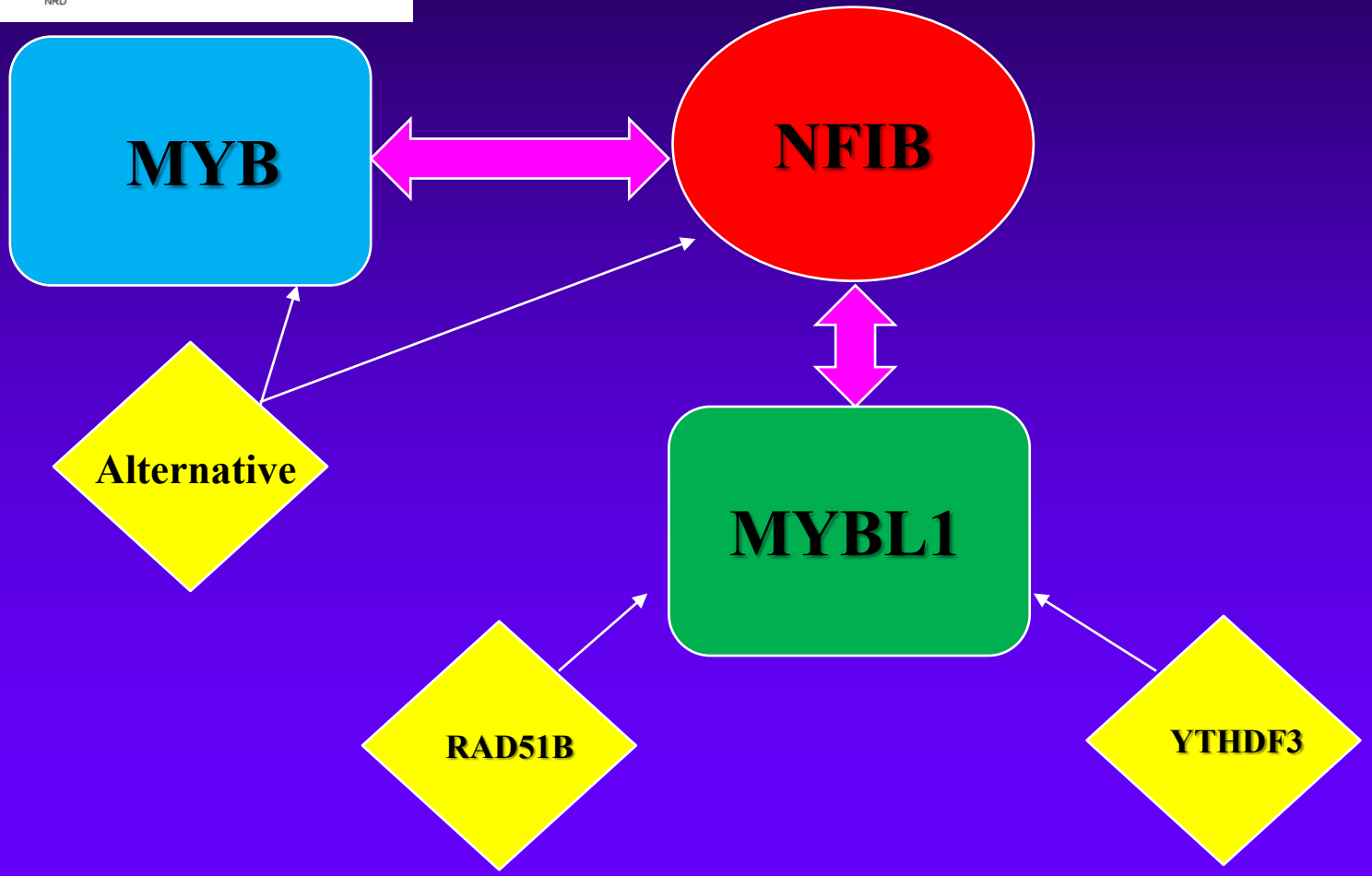
Solid Adenoid Cystic Carcinoma

More atypical, basaloid, and lacks matrix





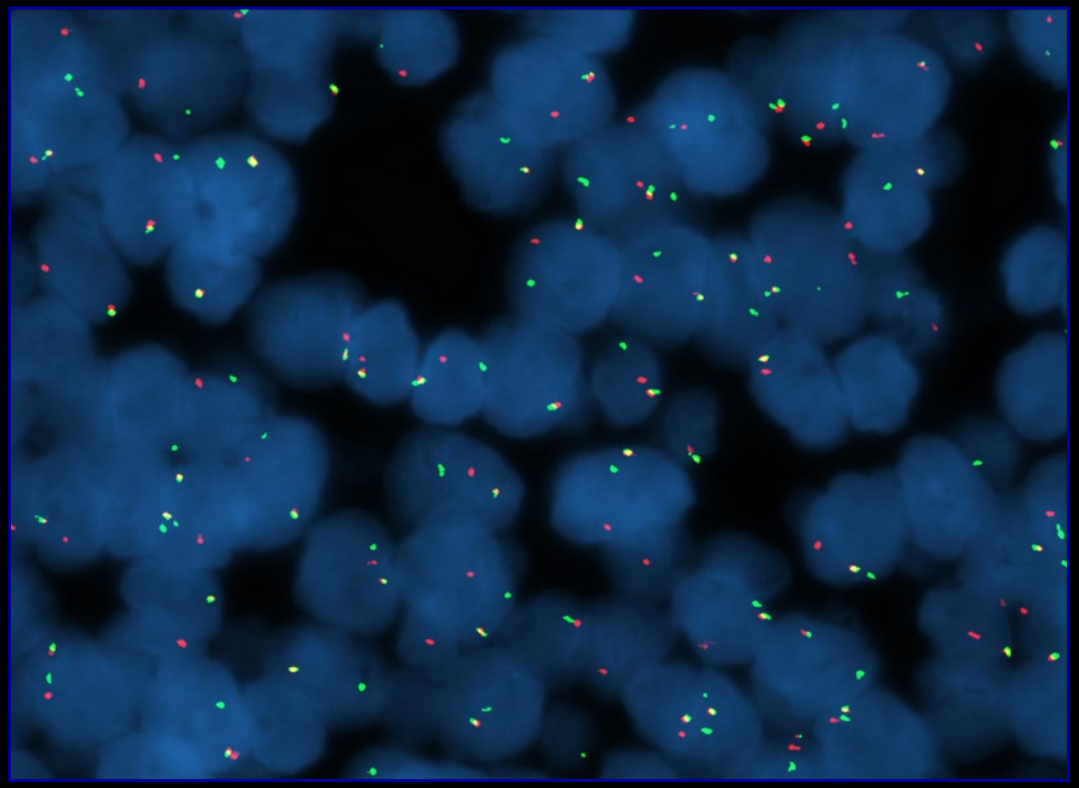
MYB-NFIB fusion



Adenoid Cystic Carcinoma: MYB Translocation by FISH

Cytogenetics:

- t(6:9) MYB oncogene-NFIB transcription factor



FISH contributed by Dr. Joaquin Garcia, Mayo Clinic

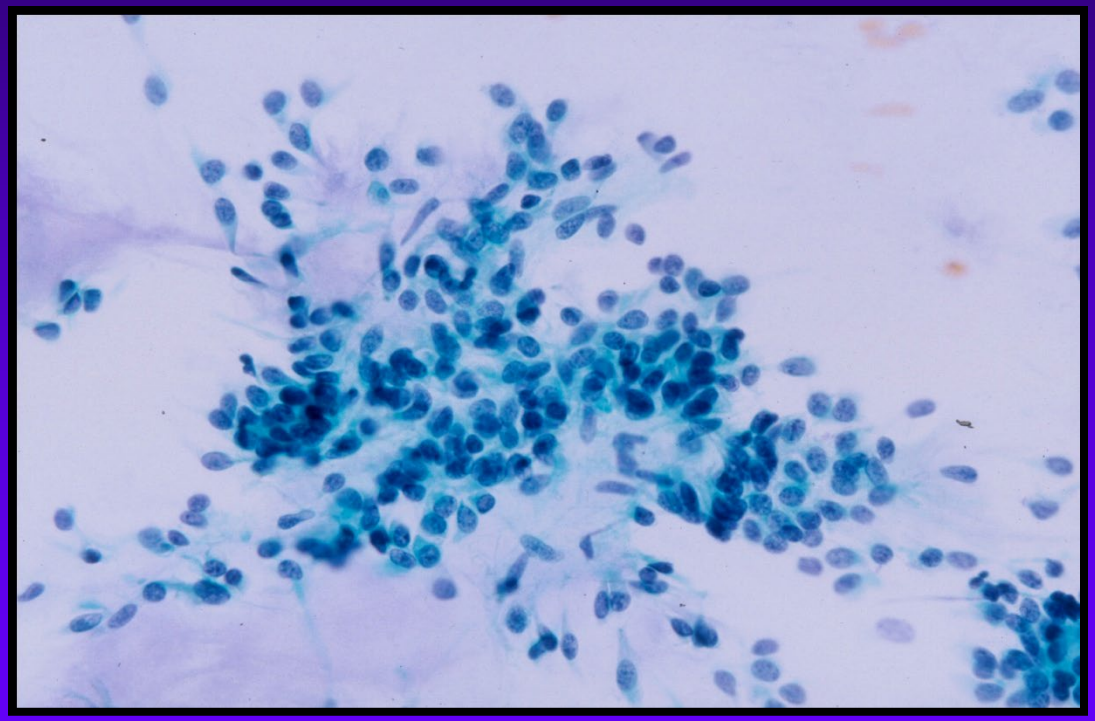
KEY POINTS

- Adenoid Cystic Cytology
  - Acellular matrix with sharp edges
  - Often cribriform pattern
  - Basaloid cells
  - Most can be classified as Suspicious or SUMP
  - MYB gene fusion

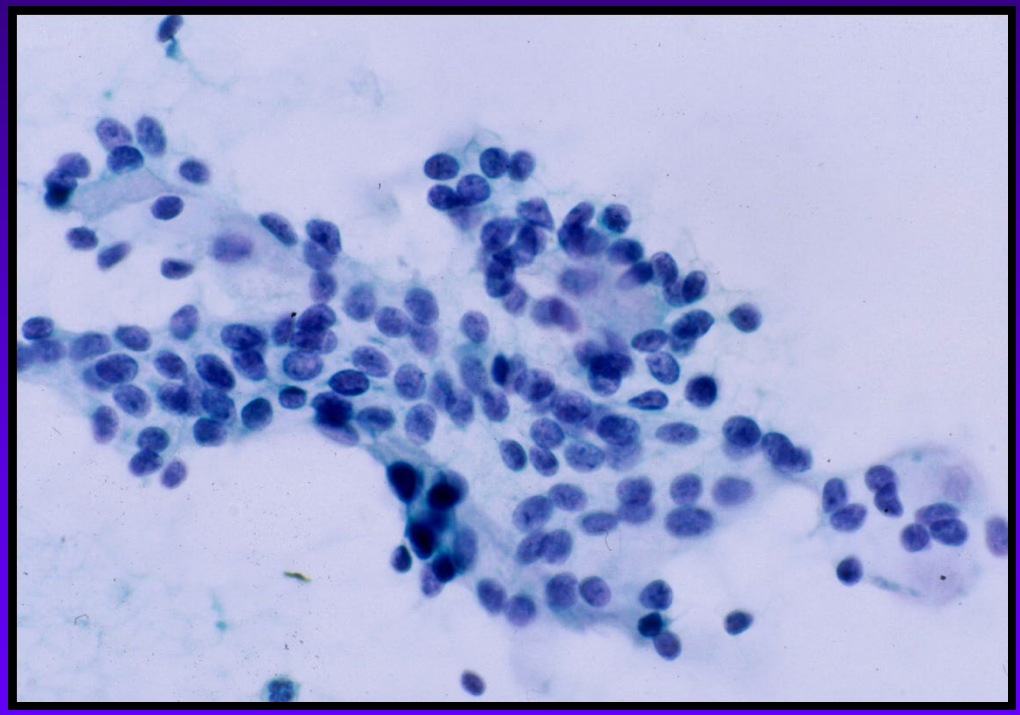
FNA Sample Preparation

Both alcohol-fixed and air-dried preparations are essential in the evaluation of matrix-containing tumors!

Pitfall: Adenoid Cystic Carcinoma vs. Pleomorphic Adenoma?

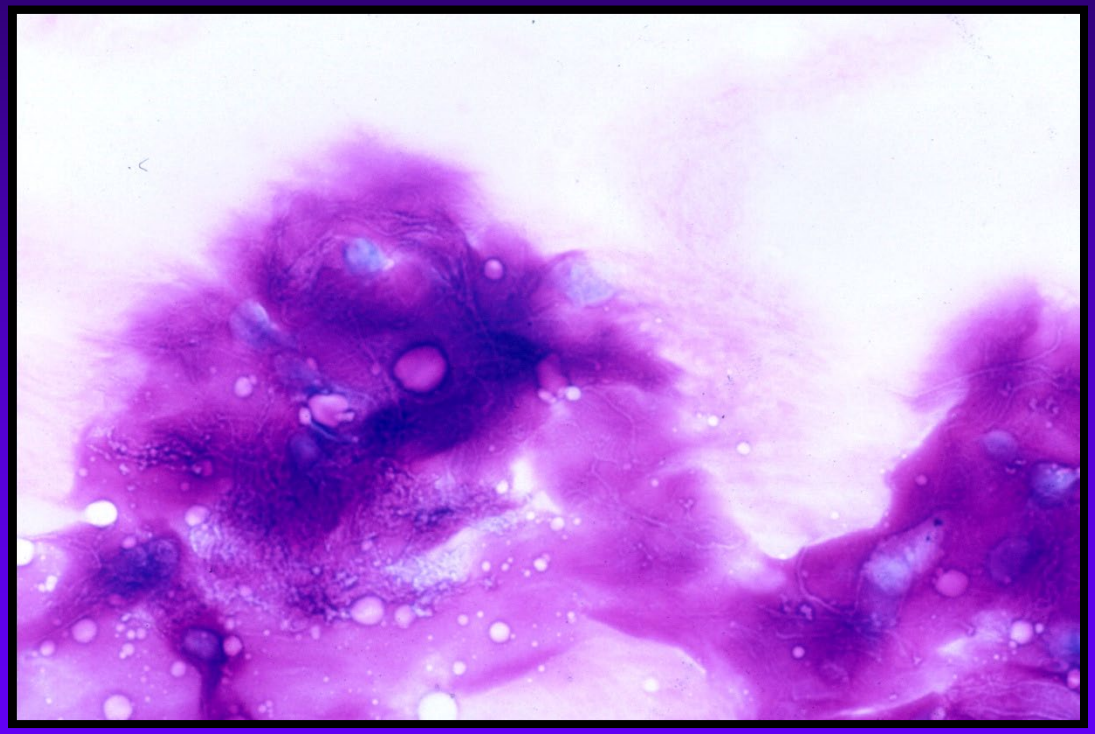


Pleomorphic adenoma

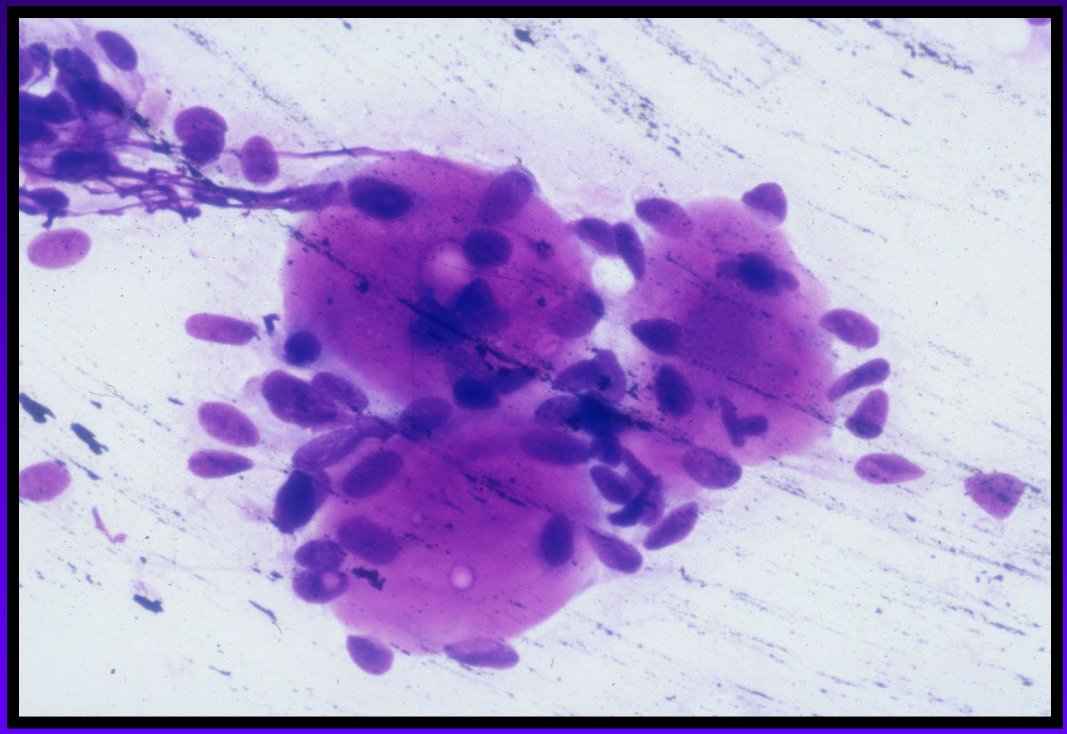


Adenoid cystic carcinoma

Pitfall: Adenoid Cystic Carcinoma vs. Pleomorphic Adenoma?



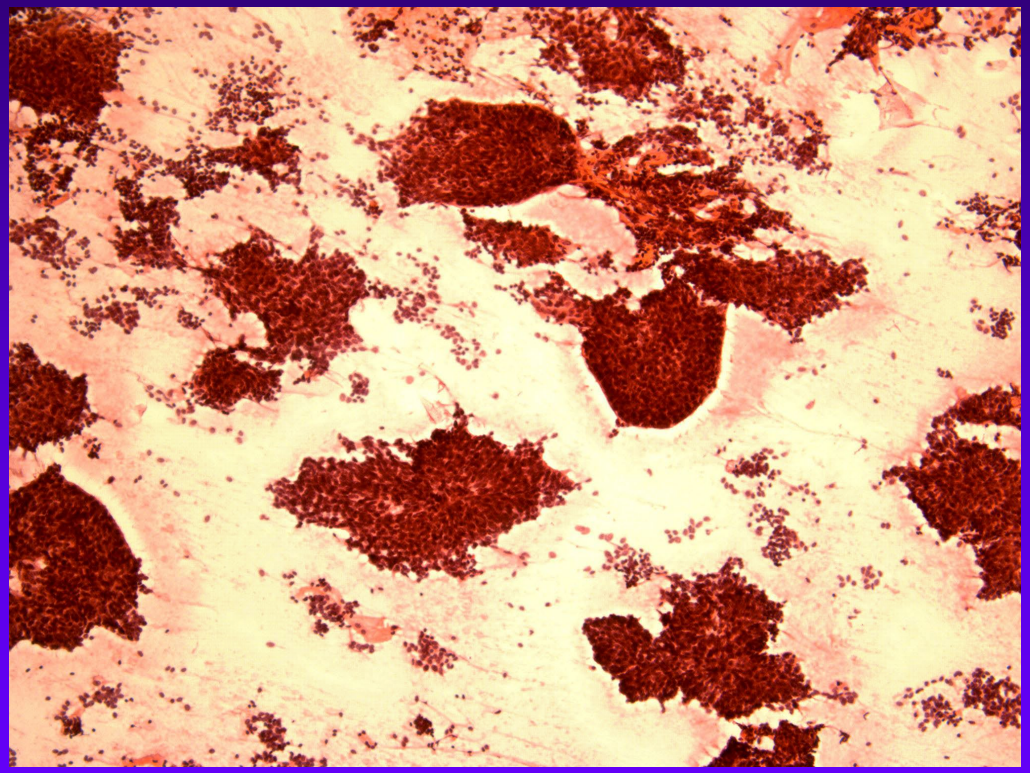
Pleomorphic adenoma

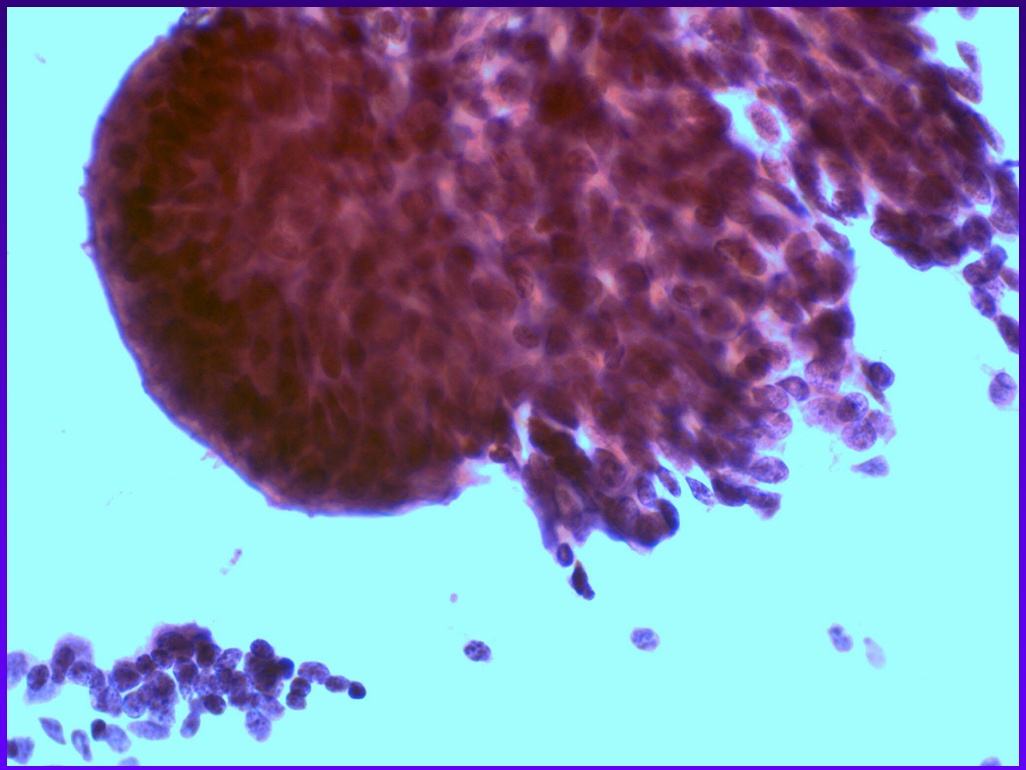


Adenoid cystic carcinoma

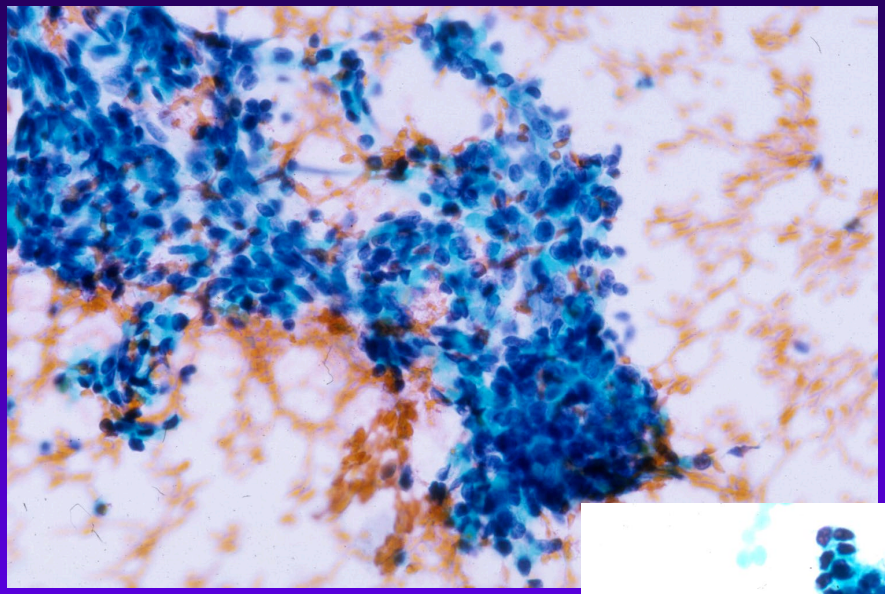
What are the features of basal cell adenoma?

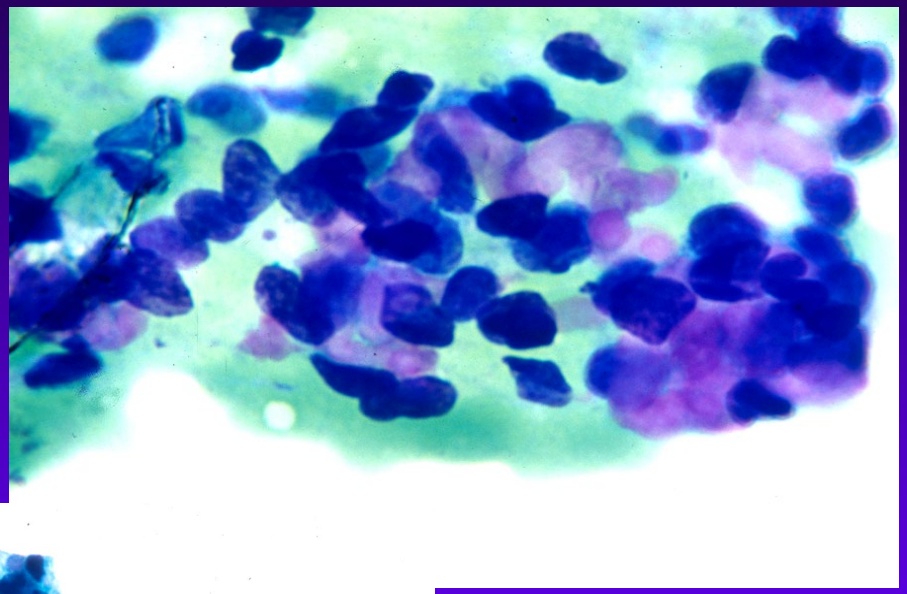
Basal Cell Adenoma:
Solid groups of basaloid cells

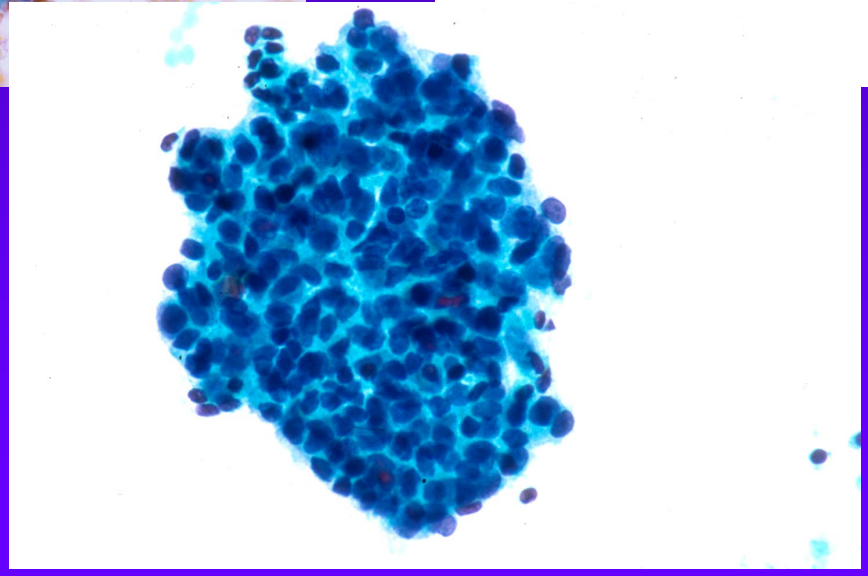




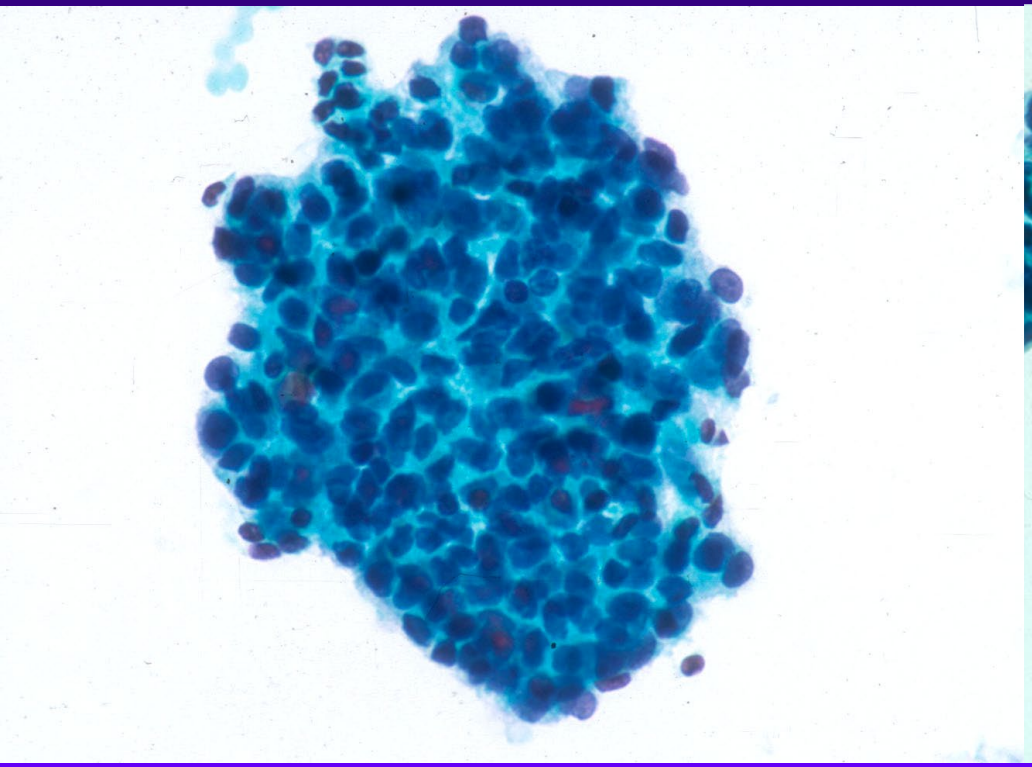
Basal Cell Adenoma is a Classic Example of Basaloid Tumors

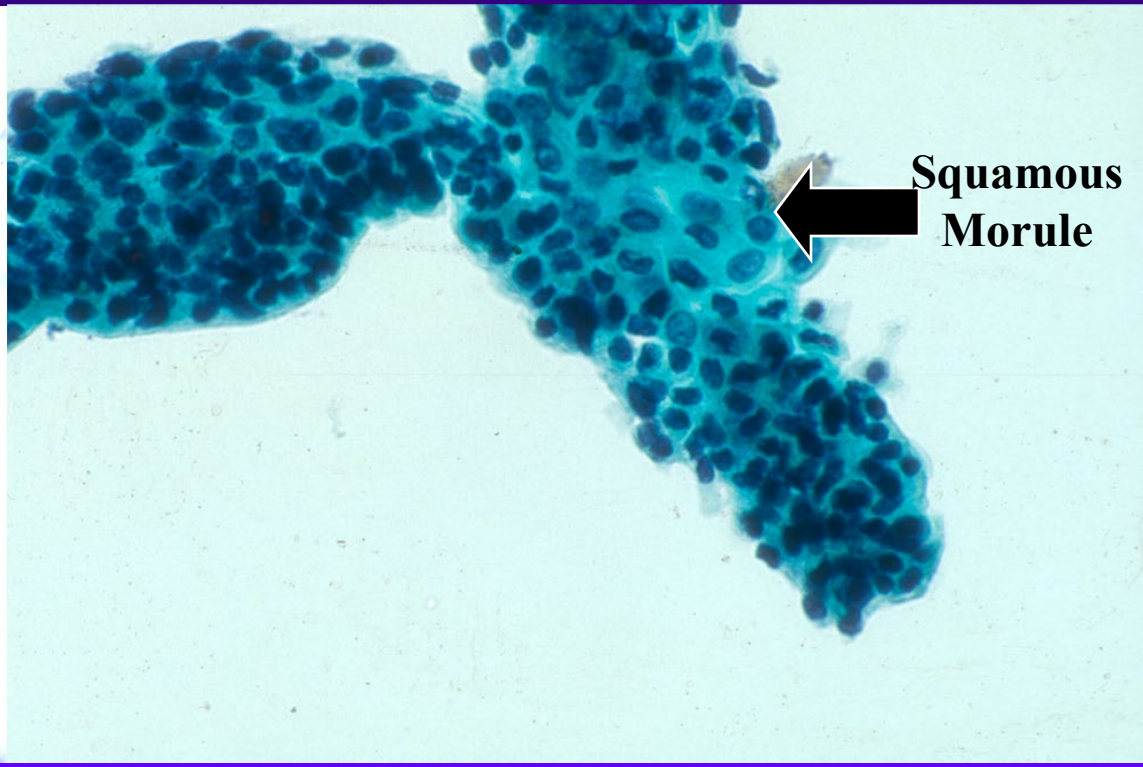




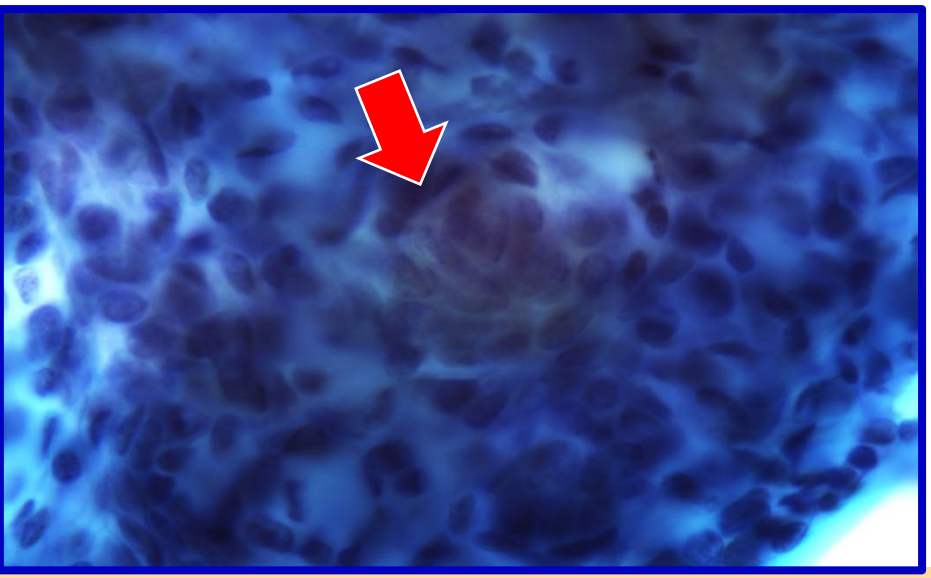


BASAL CELL ADENOMA:
Solid Type: Squamous morules are a clue

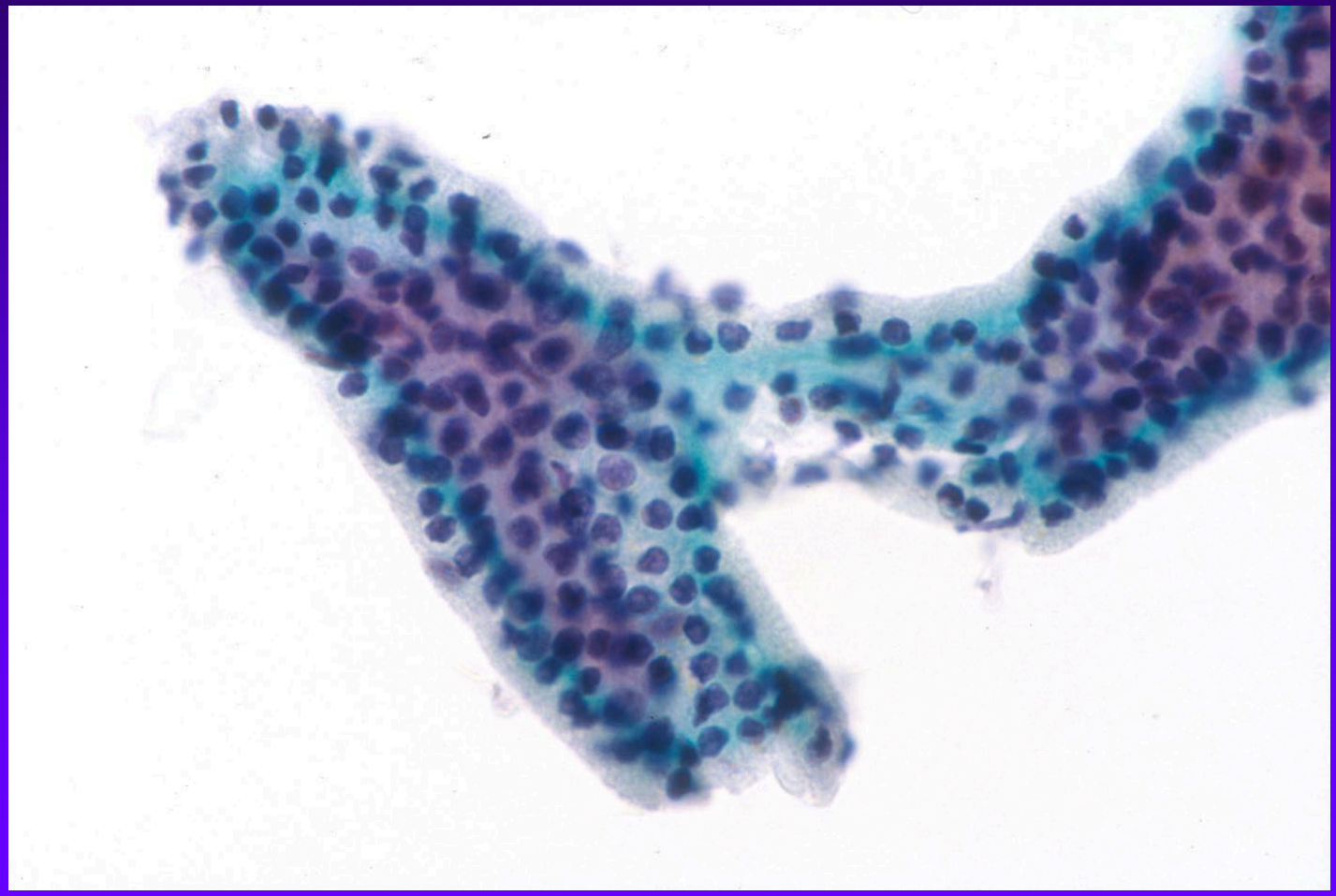




Squamous Morules

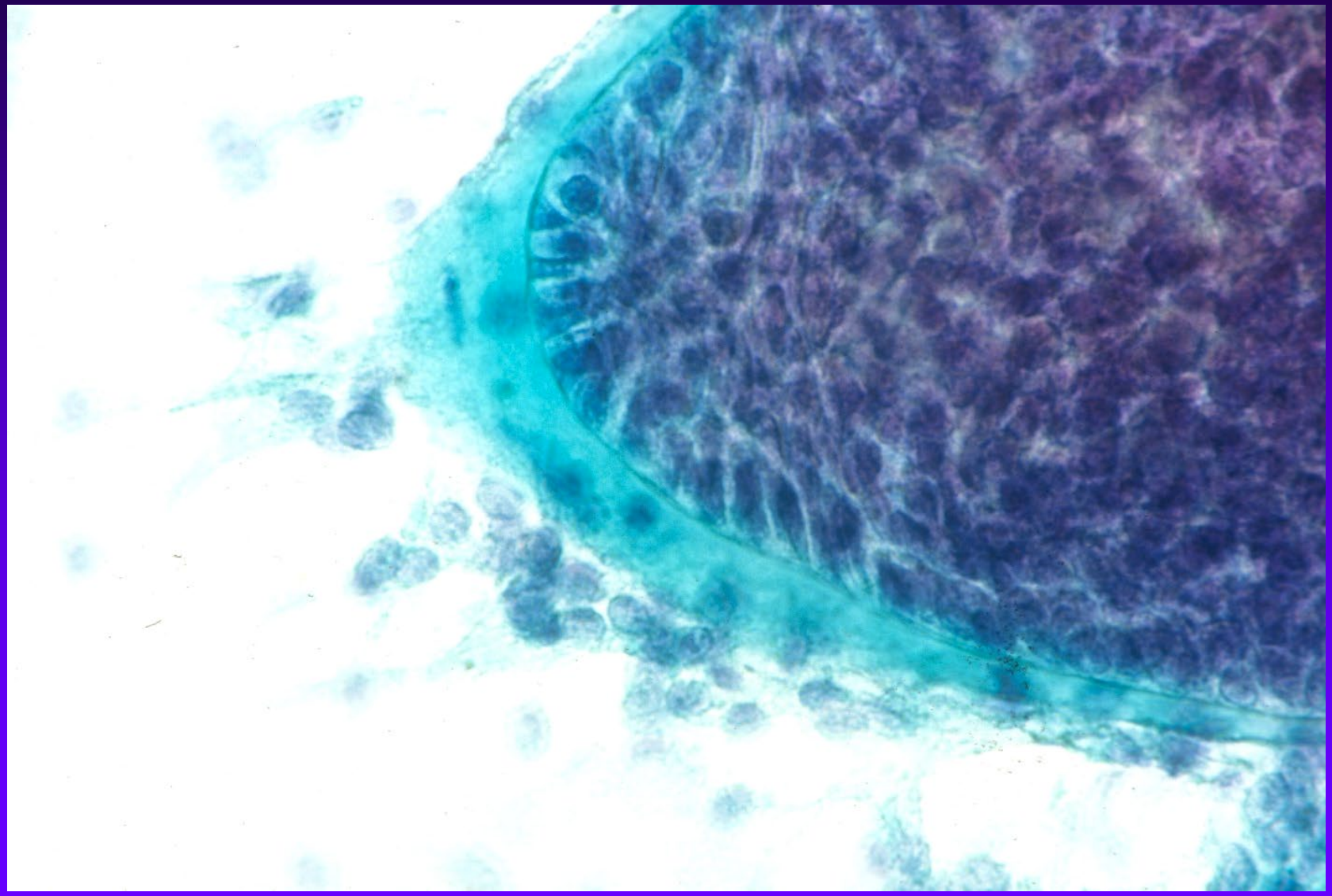


BASAL CELL ADENOMA
Peripheral ribbons of basement membrane



BASAL CELL ADENOMA:

Membranous type with prominent basement membrane

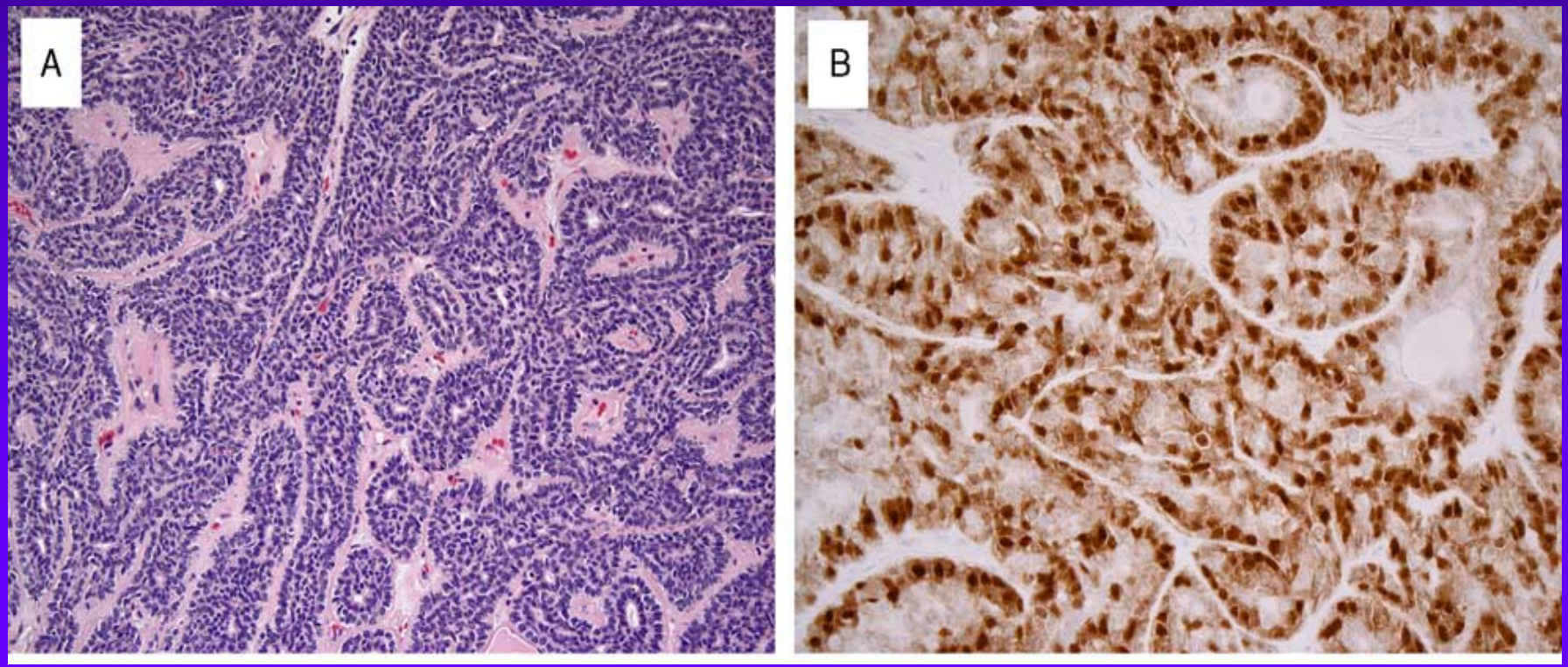


Nuclear Beta-Catenin in Basal Cell Adenoma

Jo et al. AJSP 2016;40:1143-1150.

Distinctive Patterns of *CTNNB1* (β-Catenin) Alterations in Salivary Gland Basal Cell Adenoma and Basal Cell Adenocarcinoma

Vickie Y. Jo, MD, Lynette M. Sholl, MD,*† and Jeffrey F. Krane, MD, PhD**



KEY POINTS

- Basal Cell Adenoma Cytology
  - Basaloid cells
  - Squamous morules
  - Basement membrane ribbons
  - Intercellular matrix droplets
  - Most are classified as SUMP
  - B-catenin mutations

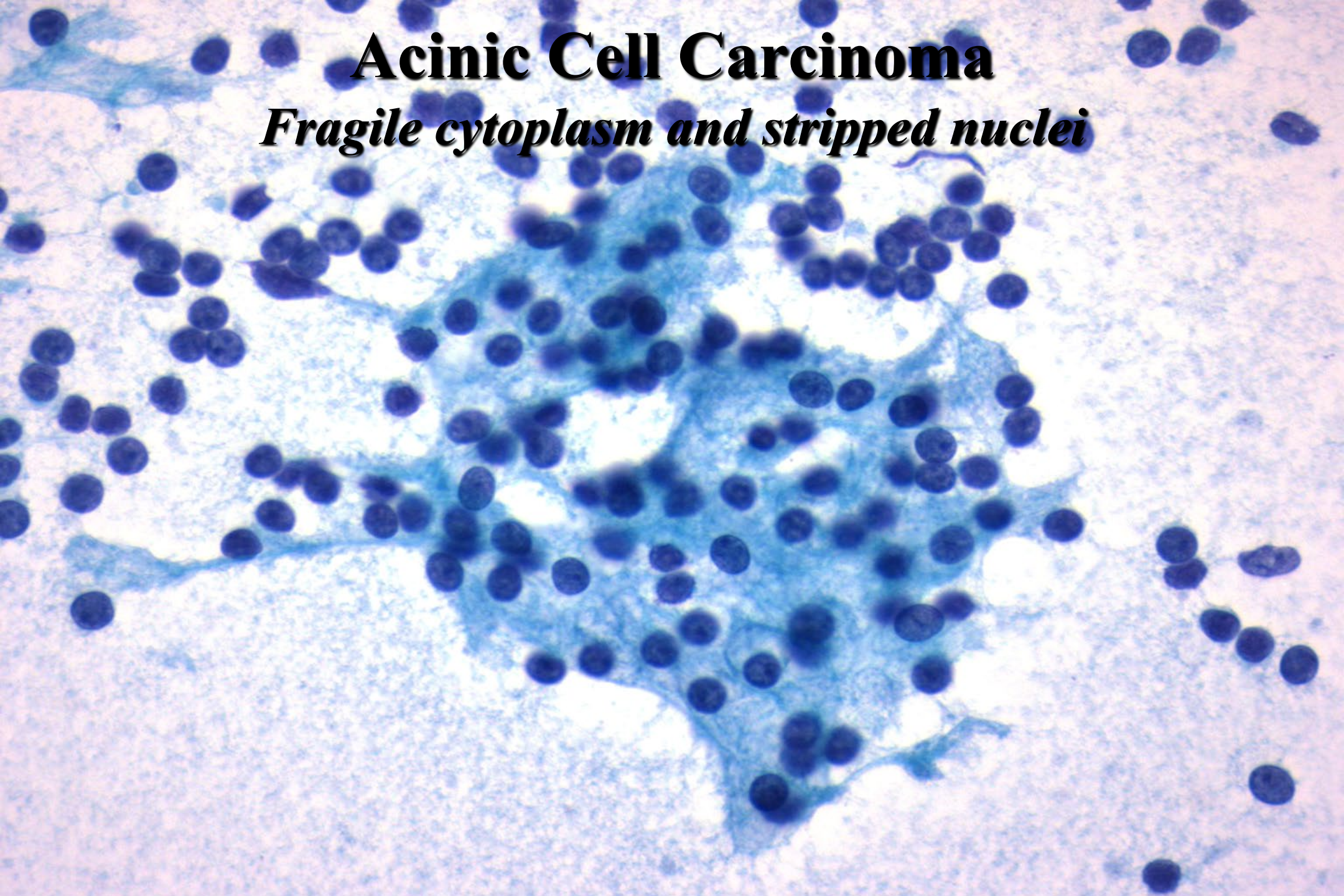
KEY POINT

Some SG FNA cases are easy to diagnosis;
Some SG FNA cases are impossible to diagnose and can be classified as SUMP.

FNA of Non-Matrix-Producing Salivary Gland Tumors

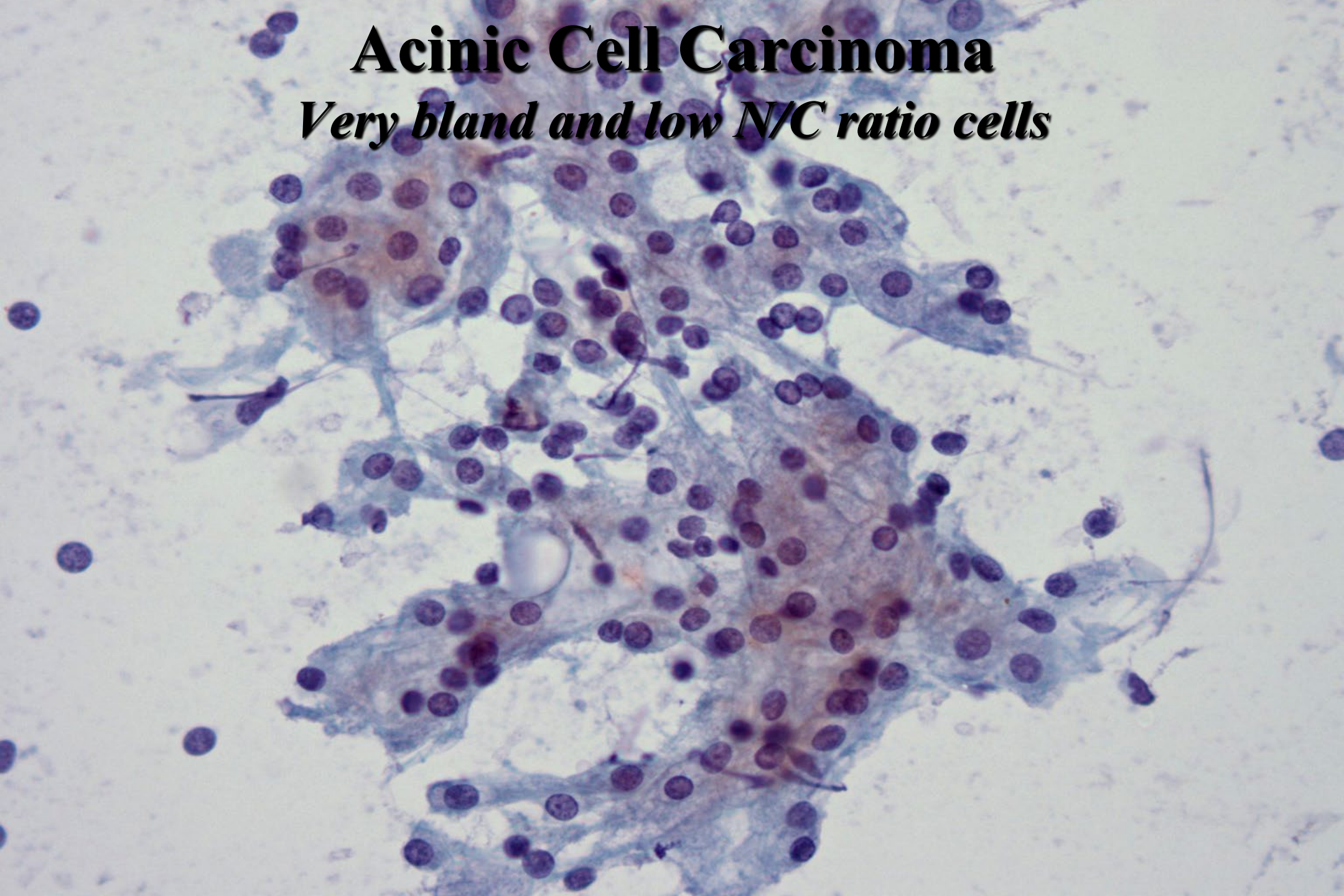
Acinic Cell Carcinoma

Fragile cytoplasm and stripped nuclei

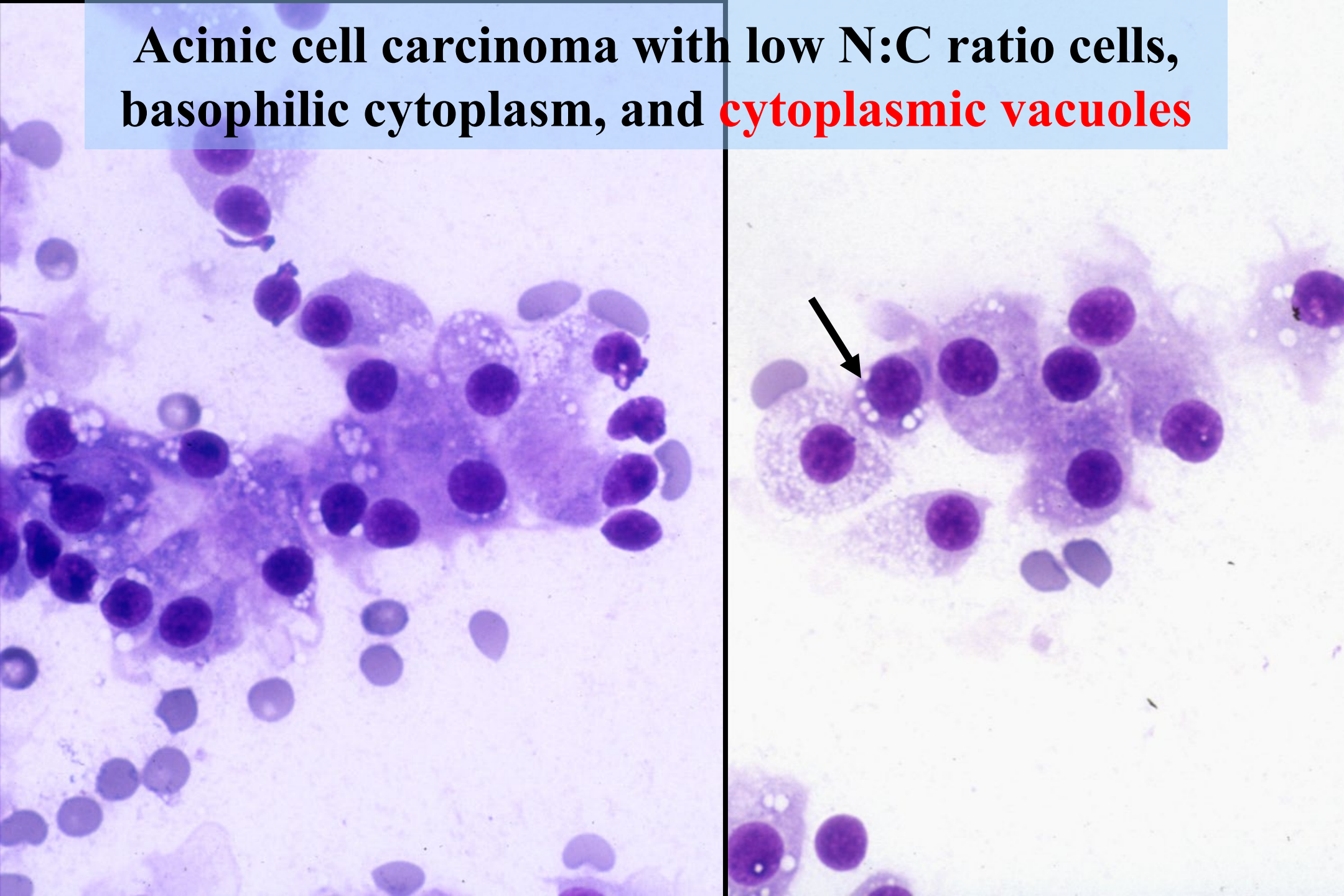


Acinic Cell Carcinoma

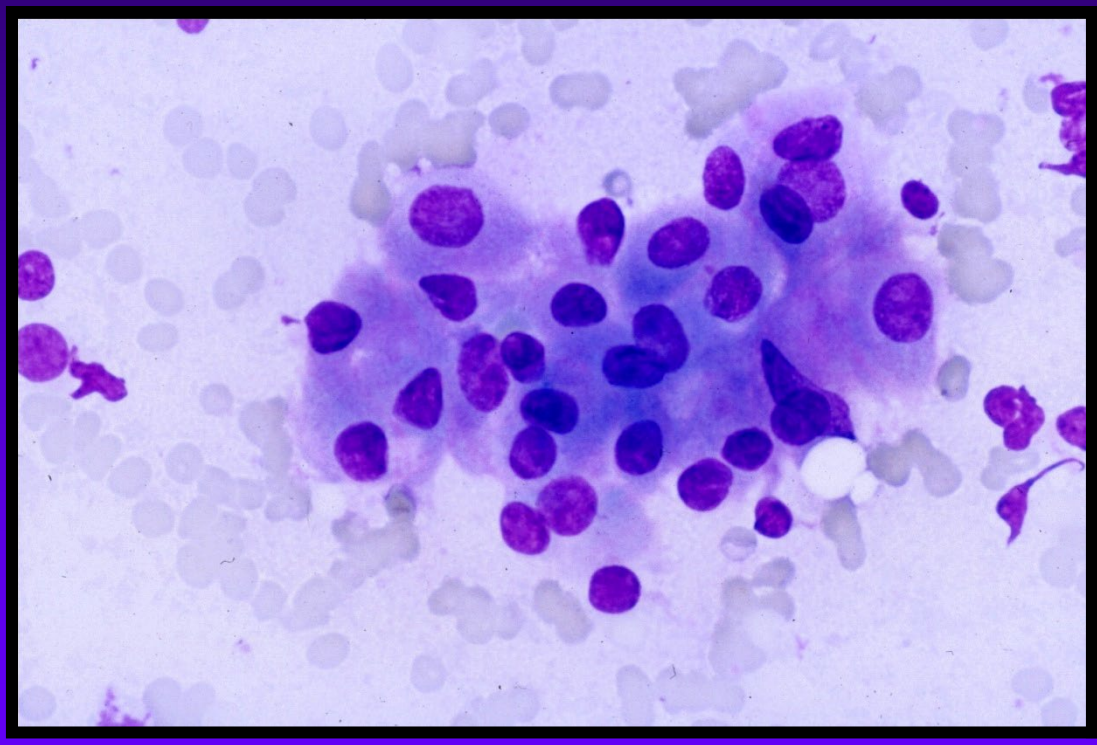
Very bland and low N/C ratio cells



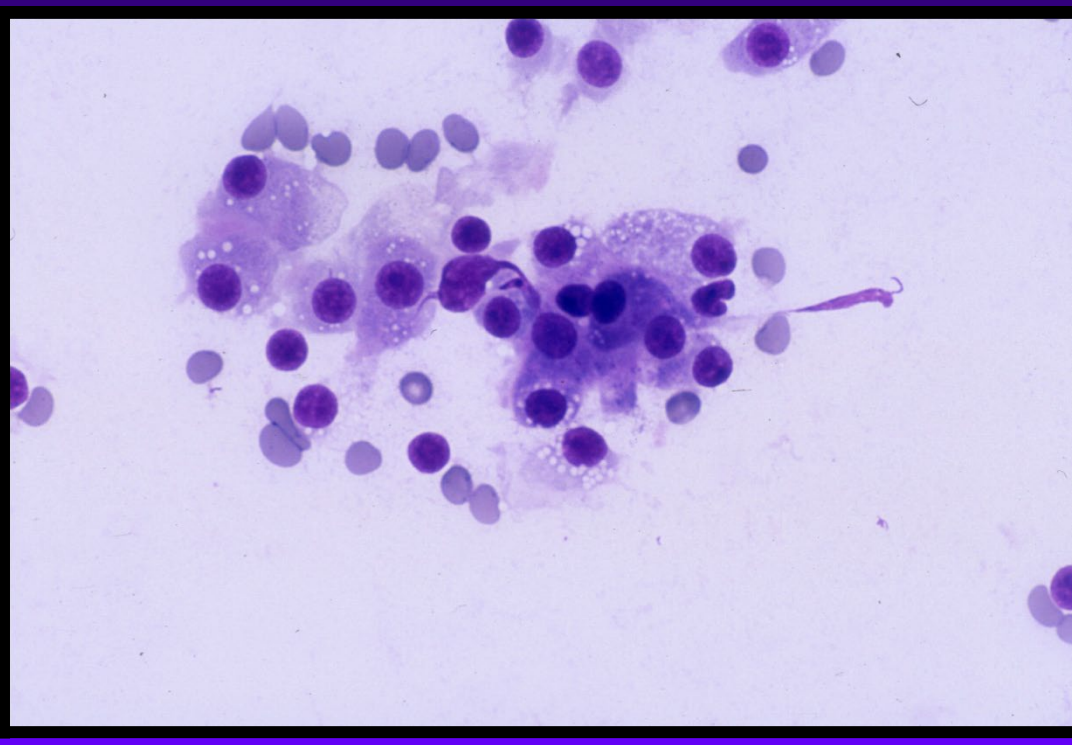
Acinic cell carcinoma with low N:C ratio cells, basophilic cytoplasm, and cytoplasmic vacuoles



Acinic Cell Carcinoma vs. Oncocytoma?



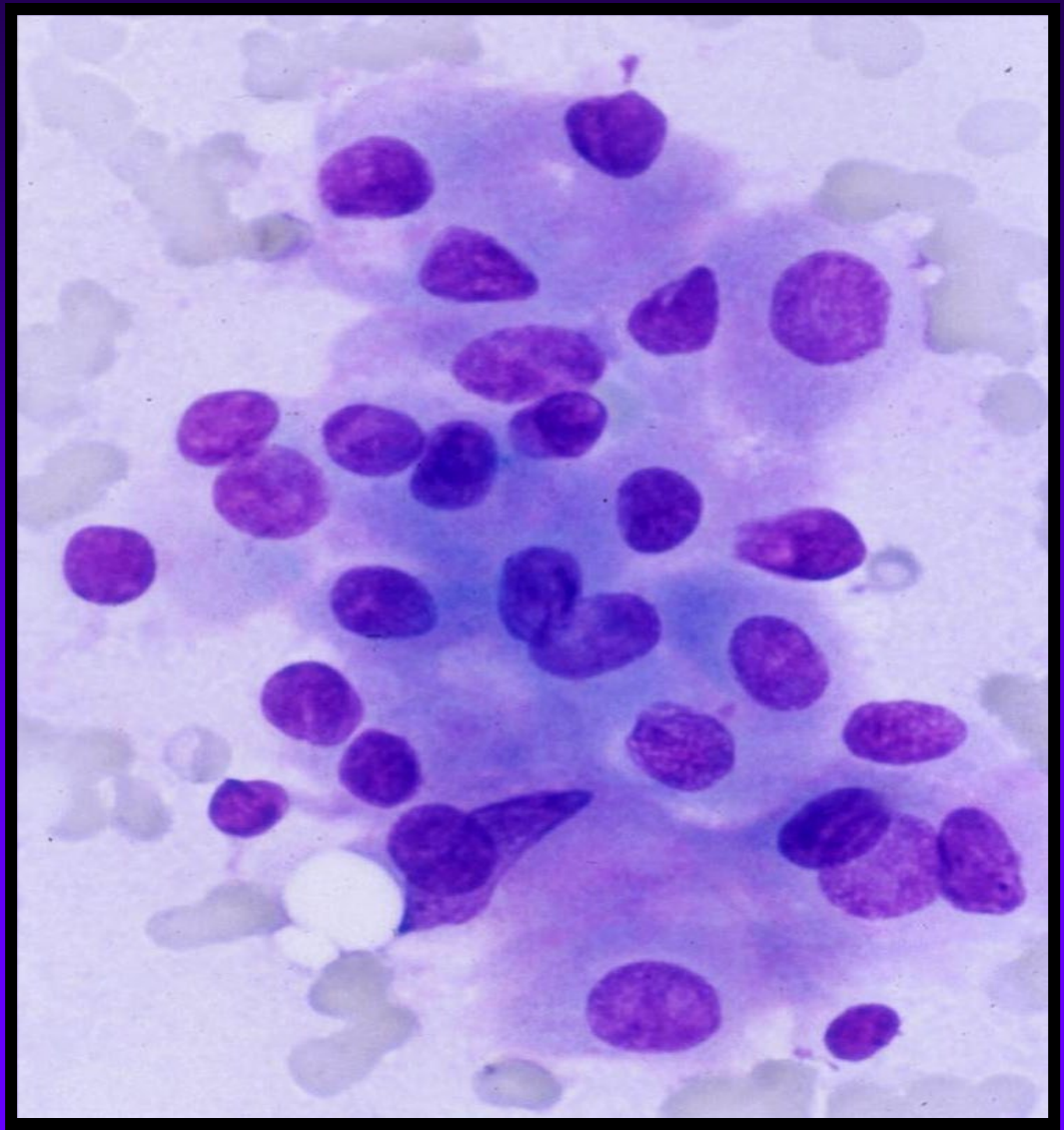
Oncocytoma

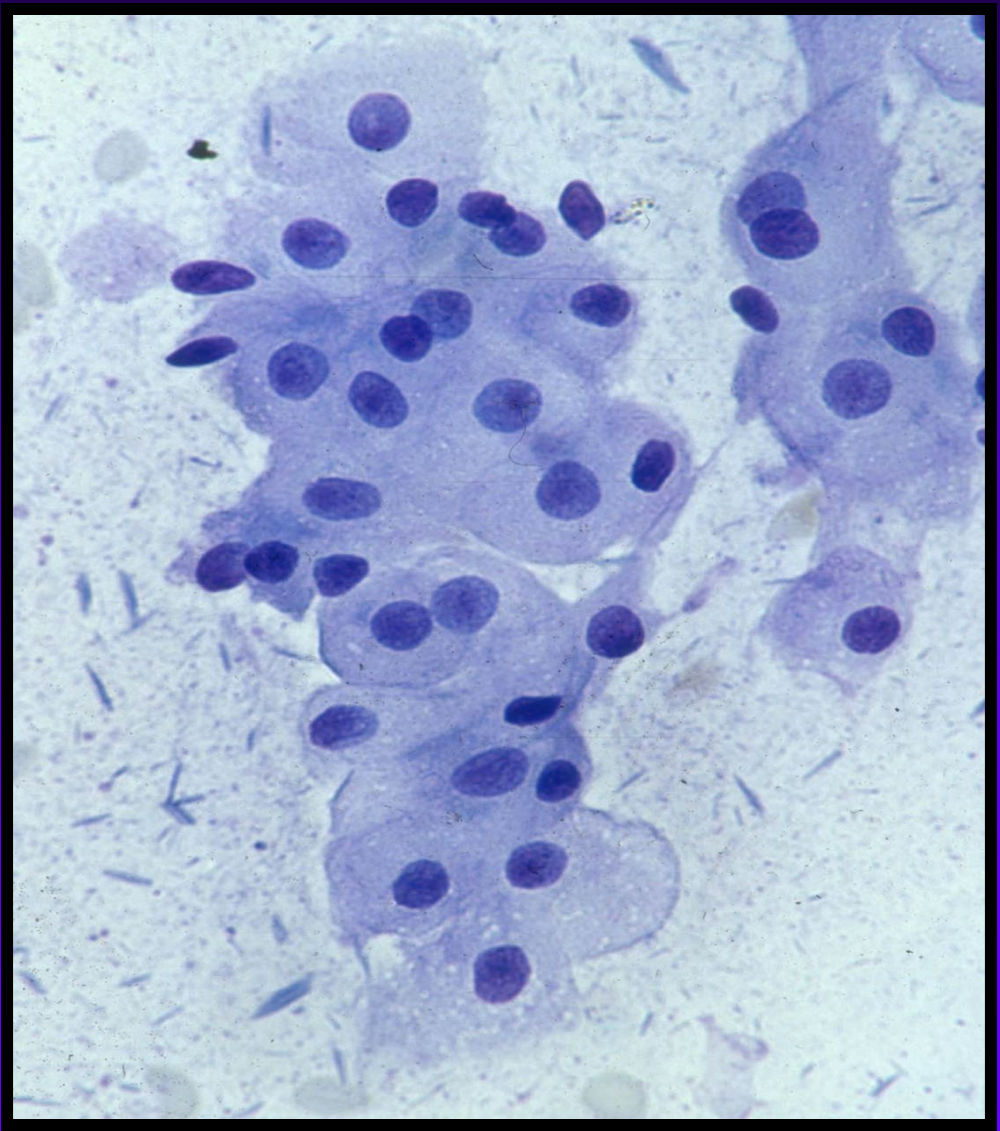


Acinic Cell Carcinoma

Oncocytoma:

Note absent cytoplasmic vacuoles, dense cytoplasm, & background crystaloids



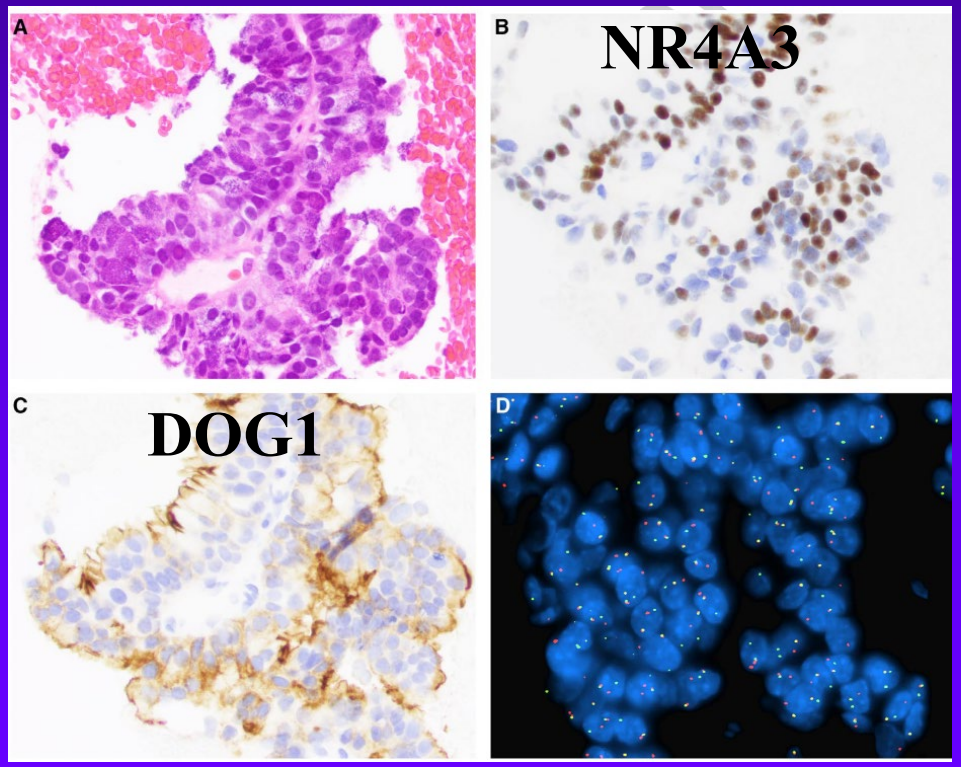


Acinic Cell Carcinoma:
Cell Block - DOG1+, SOX10+, NR4A3+

Cancer Cytopathology

Evaluation of NR4A3 Immunohistochemistry (IHC) and Fluorescence in situ Hybridization and Comparison with DOG1 IHC for Fine Needle Aspiration Diagnosis of Acinic Cell Carcinoma

JM Skaugen, RR Seethala, SI Chiosea, MS Landau. Cancer Cytopathology 2020

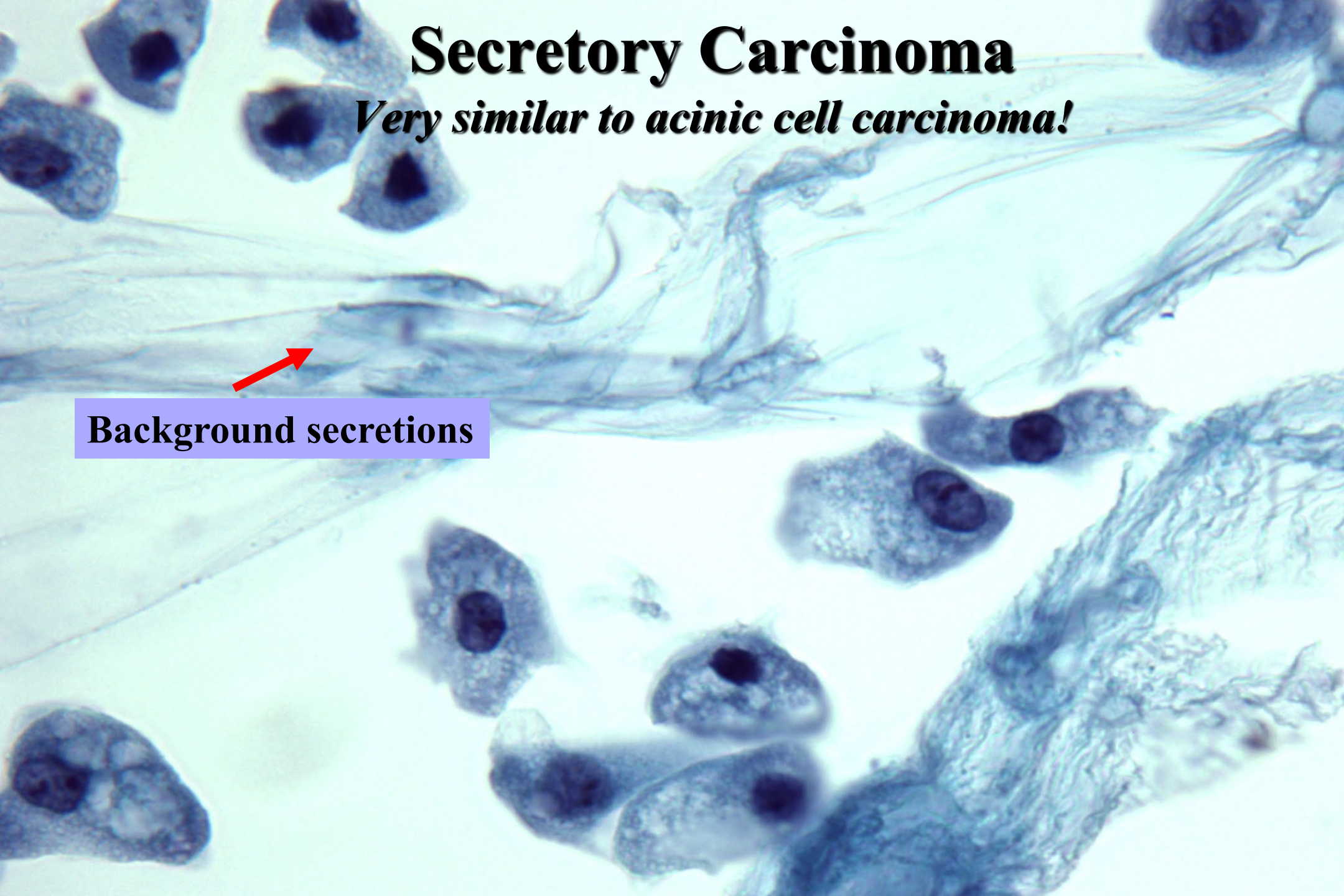


KEY POINTS

- Acinic Cell Carcinoma Cytology
  - Bland, low N/C ratio cells
  - Fragile vacuolated cytoplasm
  - Basophilic appearance
  - Background stripped nuclei
  - Most will be classified as SUSPICIOUS by FNA
  - NR4A3 positive

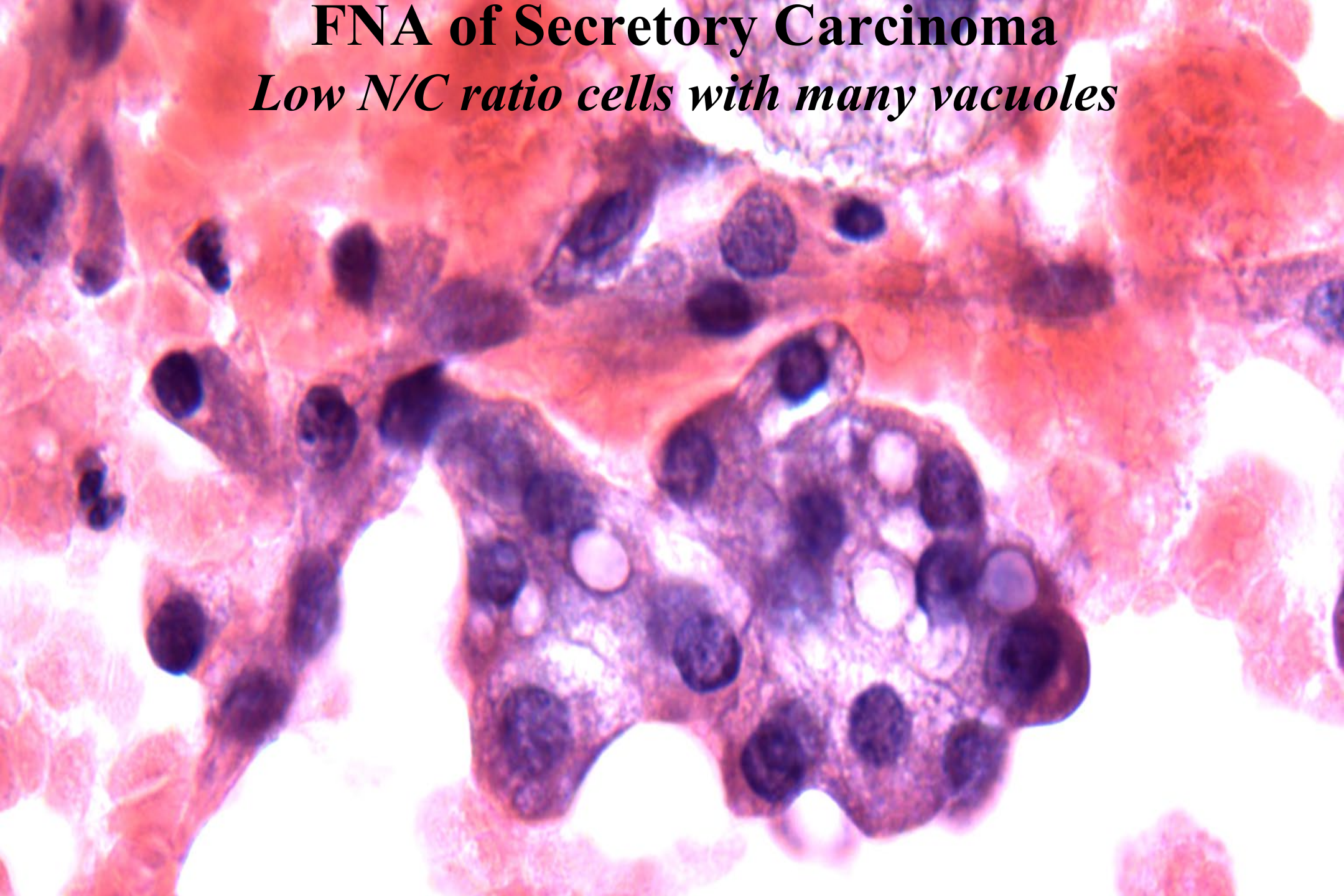
Secretory Carcinoma

Very similar to acinic cell carcinoma!



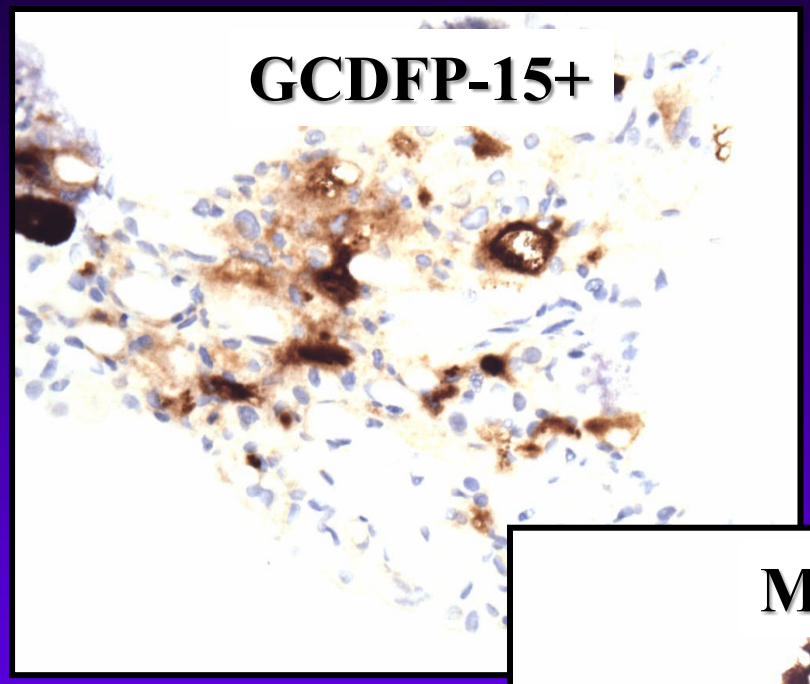
FNA of Secretory Carcinoma

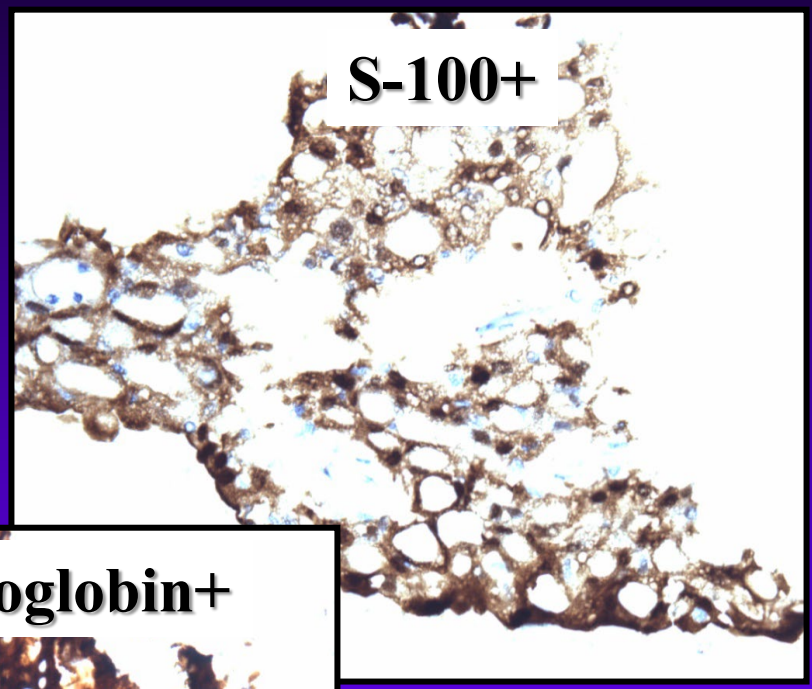
Low N/C ratio cells with many vacuoles

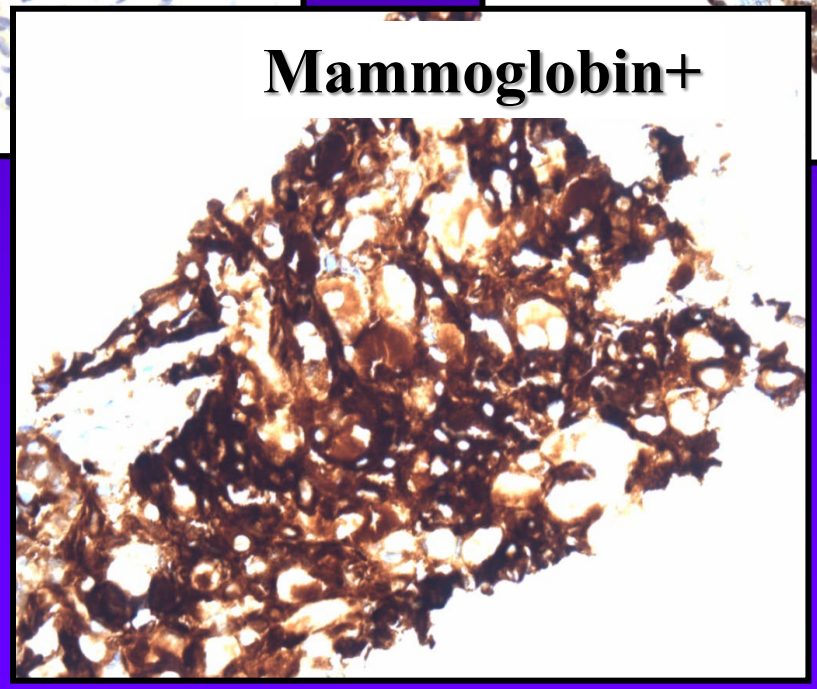


Secretory Carcinoma: Immunohistochemical Studies

GATA-3+, S-100+, Mammaglobin+, GCDFP-15+







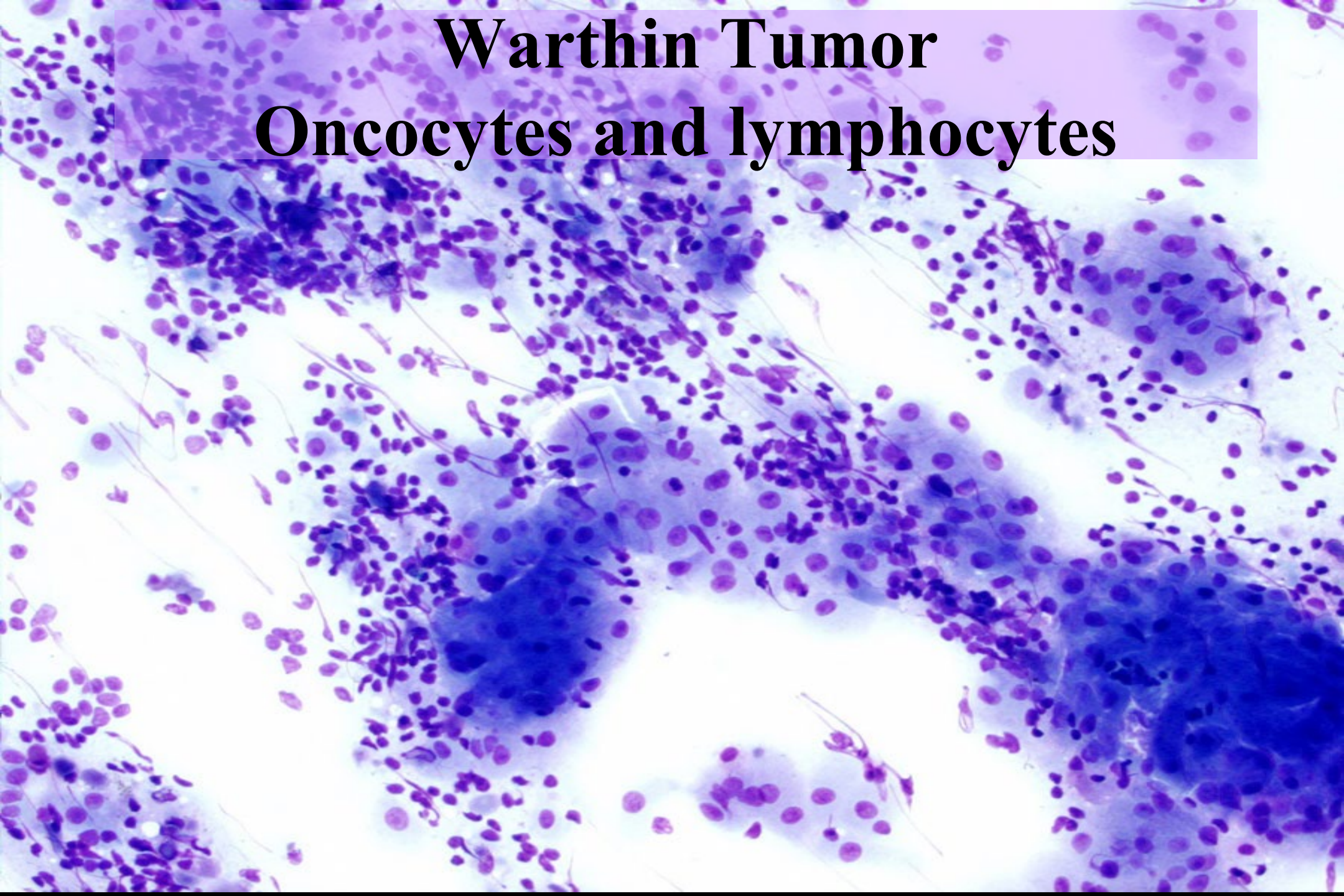
KEY POINTS

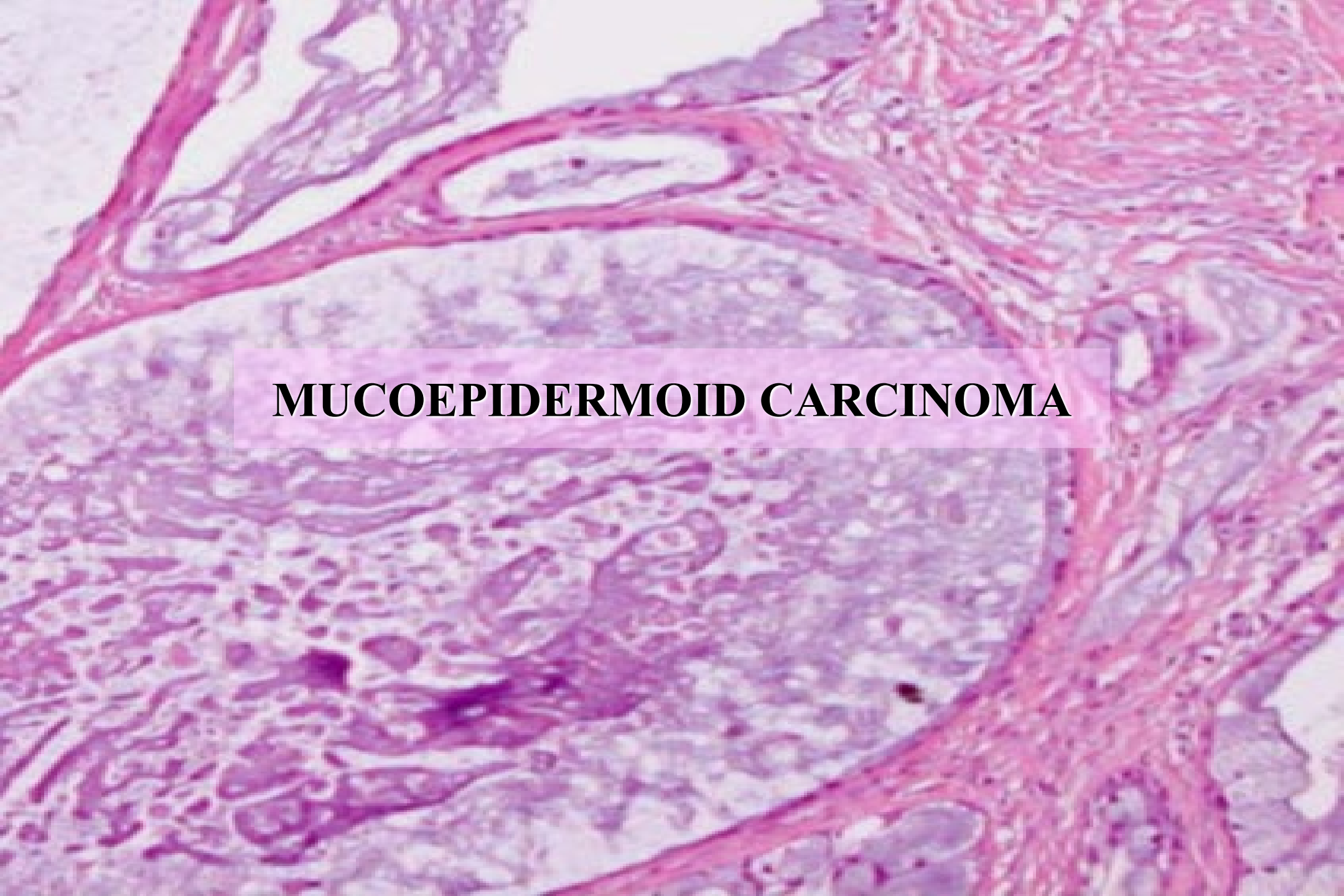
- **Secretory Carcinoma Cytology**
  - **Bland appearance**
  - **Highly vacuolated, low N/C ratio cells**
  - **Background secretions**
  - **Most will be classified as SUSPICIOUS or SUMP**
  - **Mammaglobin positive/ NTRK fusions**

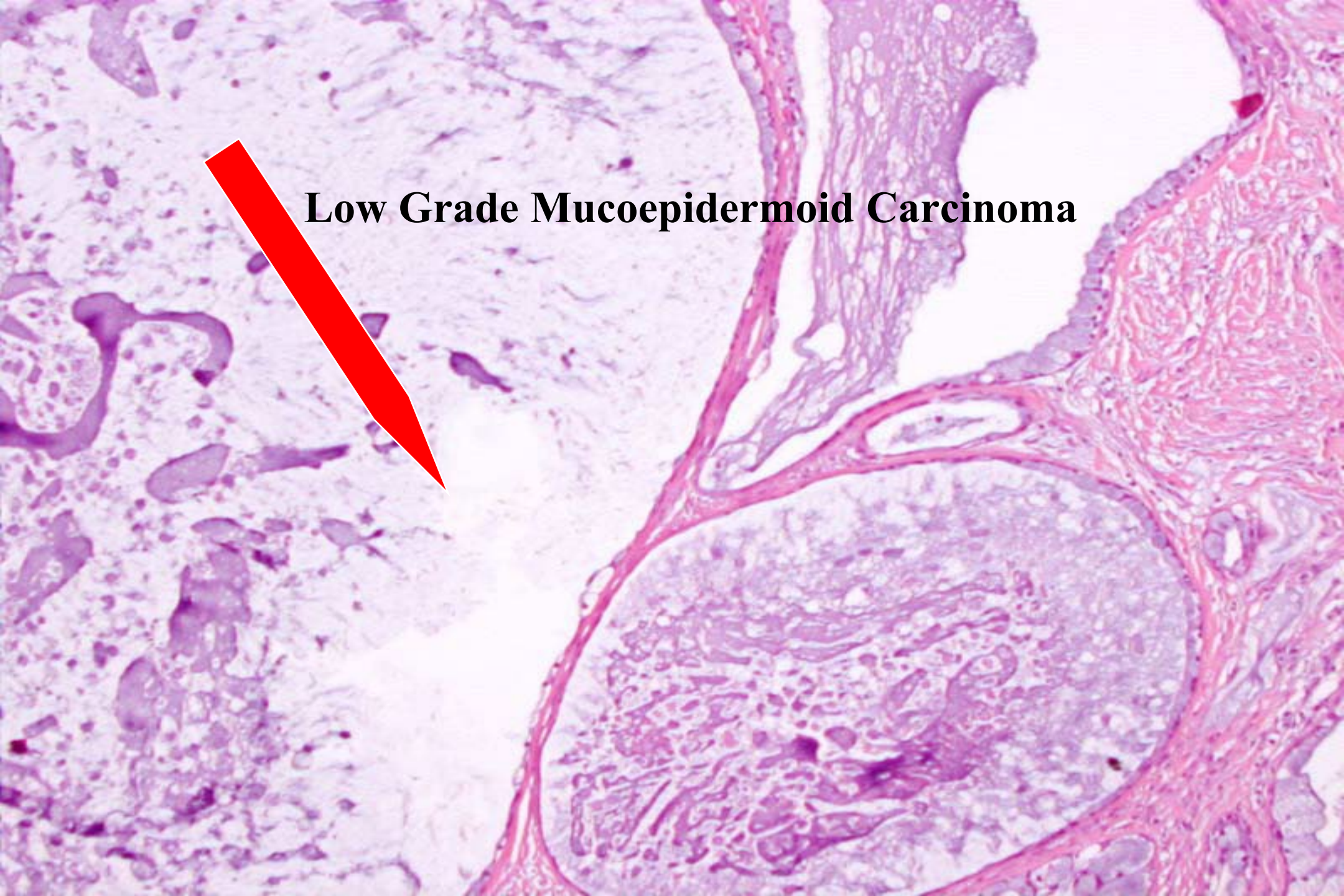
What are the 3 cytologic features of Warthin Tumor???


Warthin Tumor:
Oncocytes, lymphocytes, and background debris
Most are classified as BENIGN by FNA

Warthin Tumor
Oncocytes and lymphocytes

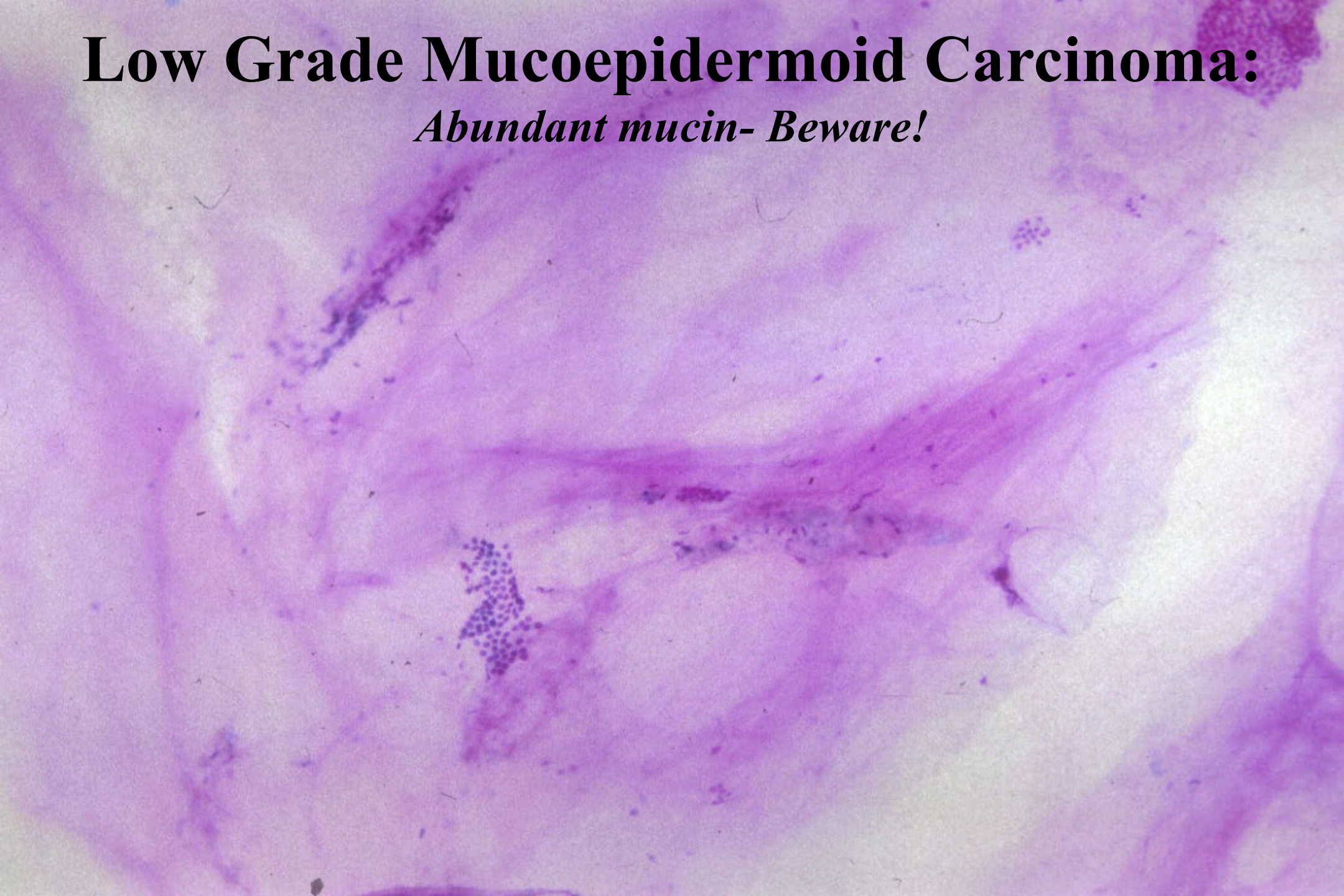



MUCOEPIDERMOID CARCINOMA


Low Grade Mucoepidermoid Carcinoma

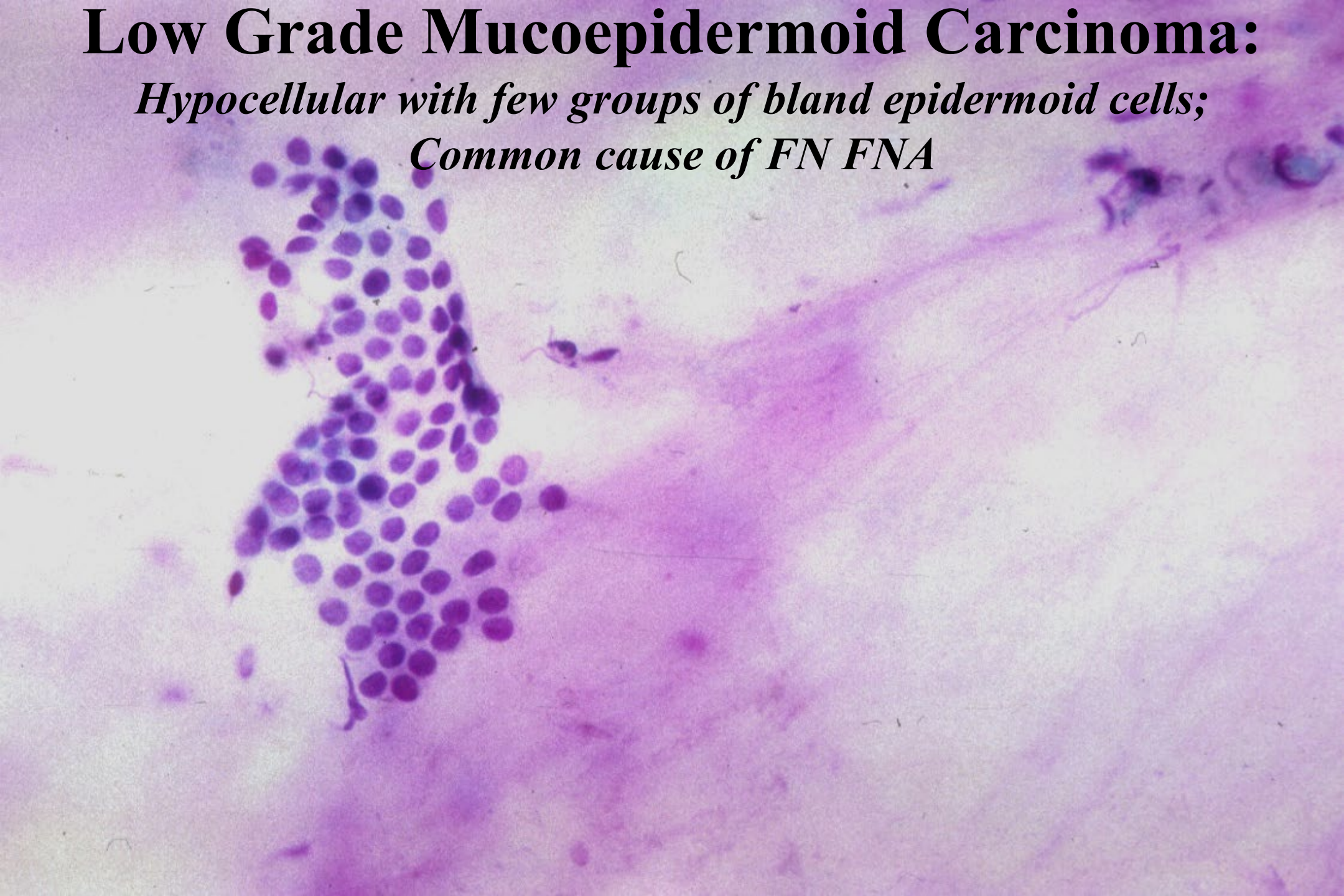
Low Grade Mucoepidermoid Carcinoma:

Abundant mucin- Beware!



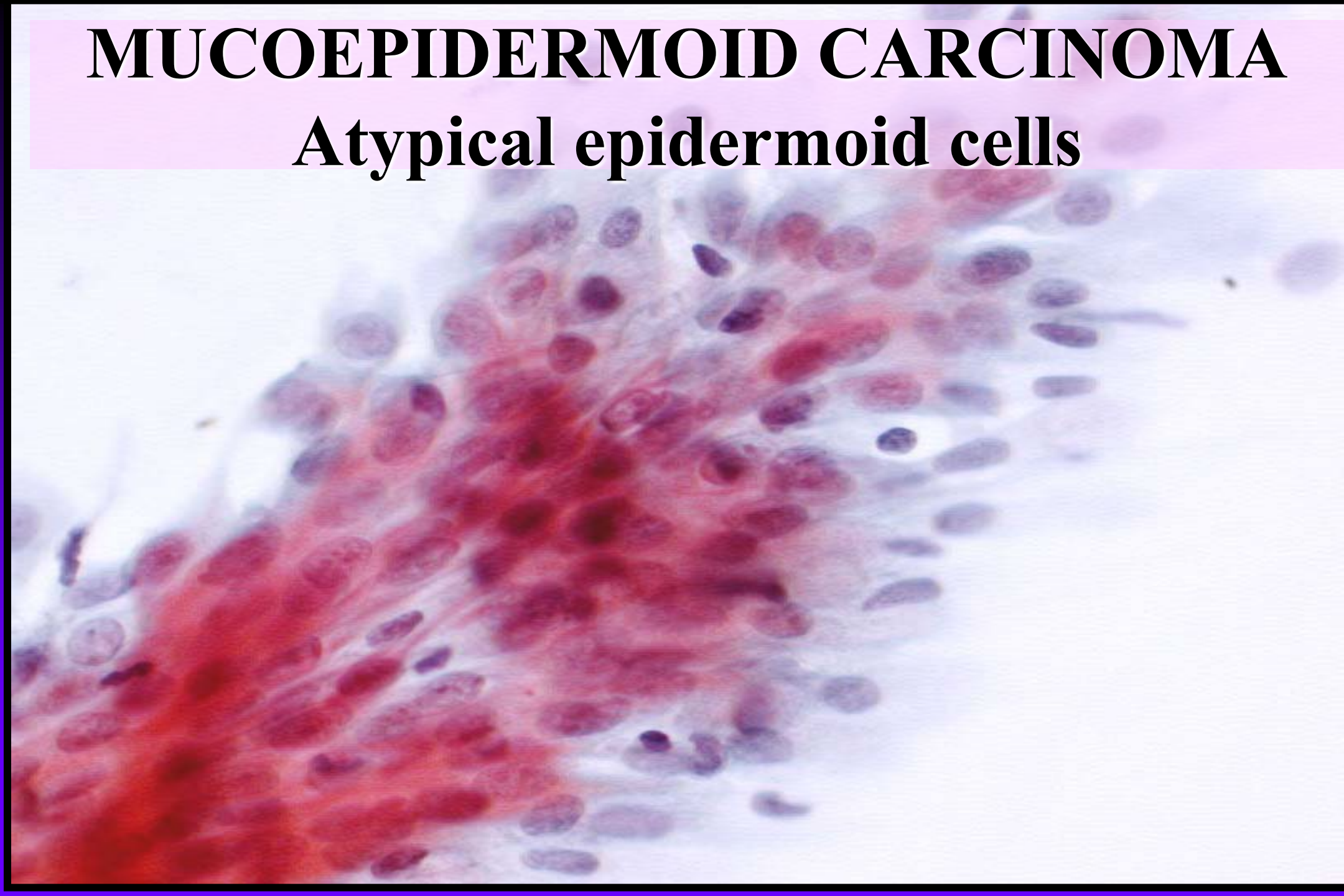
Low Grade Mucoepidermoid Carcinoma:

Hypocellular with few groups of bland epidermoid cells;
Common cause of FN FNA

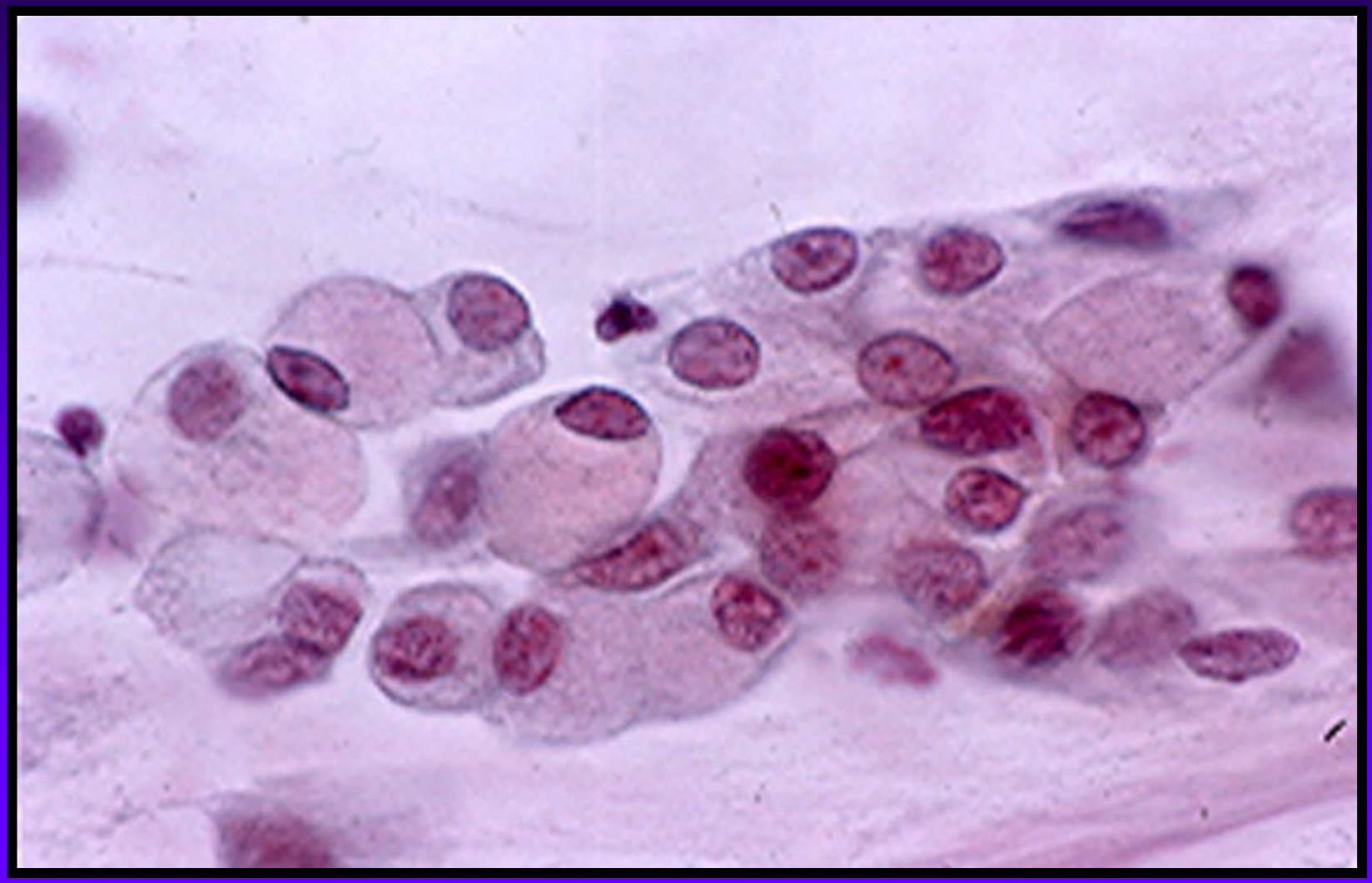


MUCOEPIDERMOID CARCINOMA

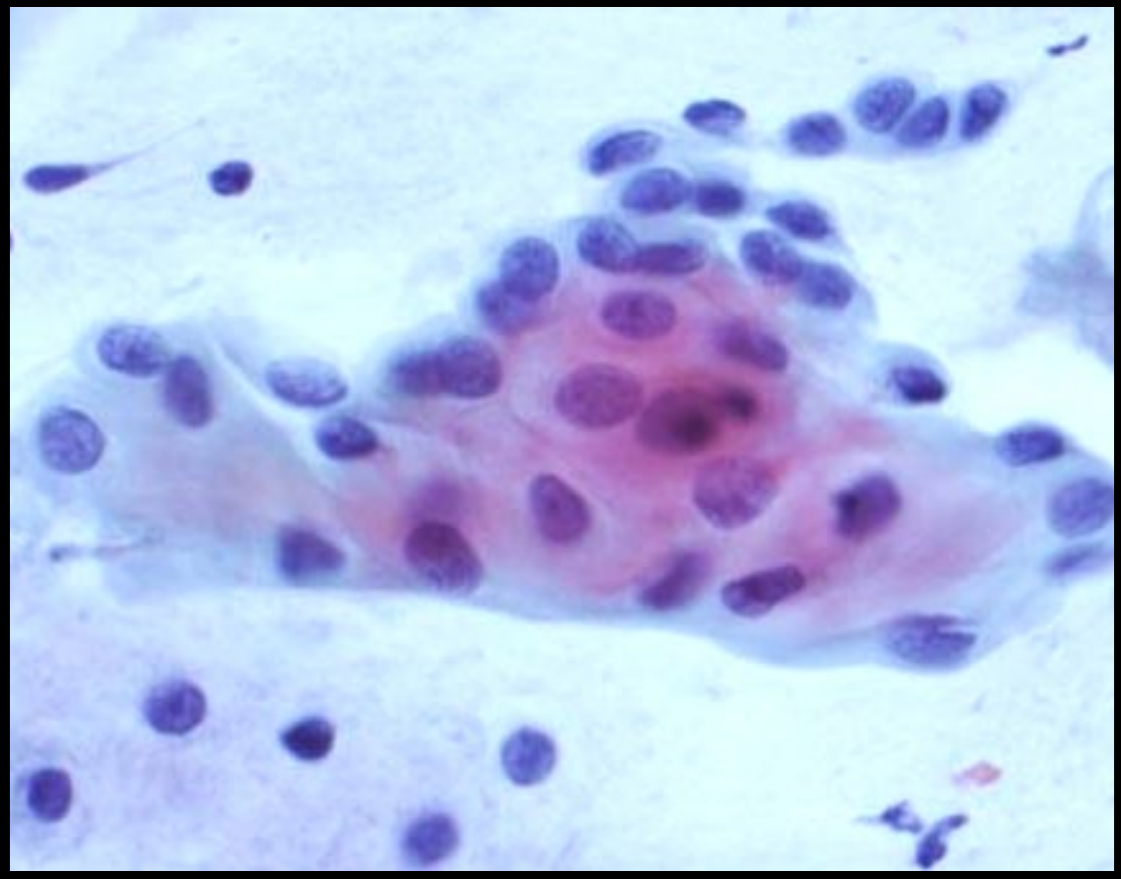
Atypical epidermoid cells

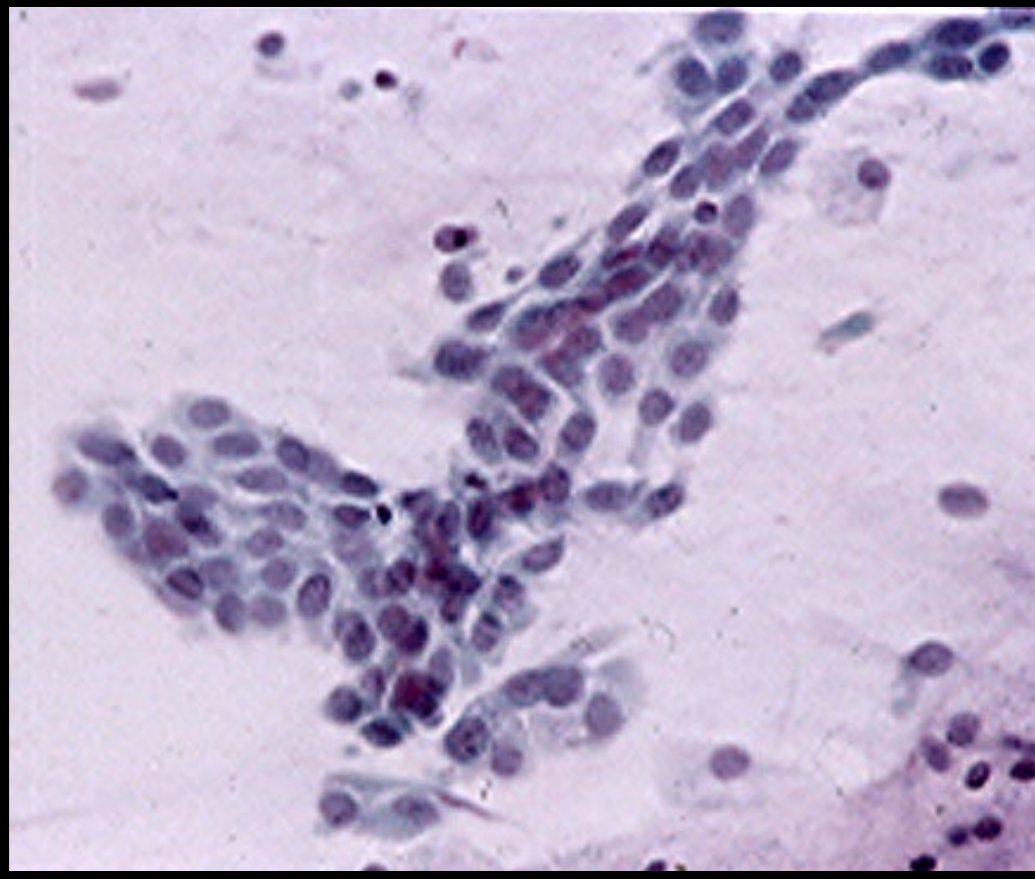


Low-Grade Mucoepidermoid Carcinoma: Mucus-Containing Epithelial Cells



Mucoepidermoid Carcinoma: Intermediate Cells





Mucoepidermoid Carcinoma:

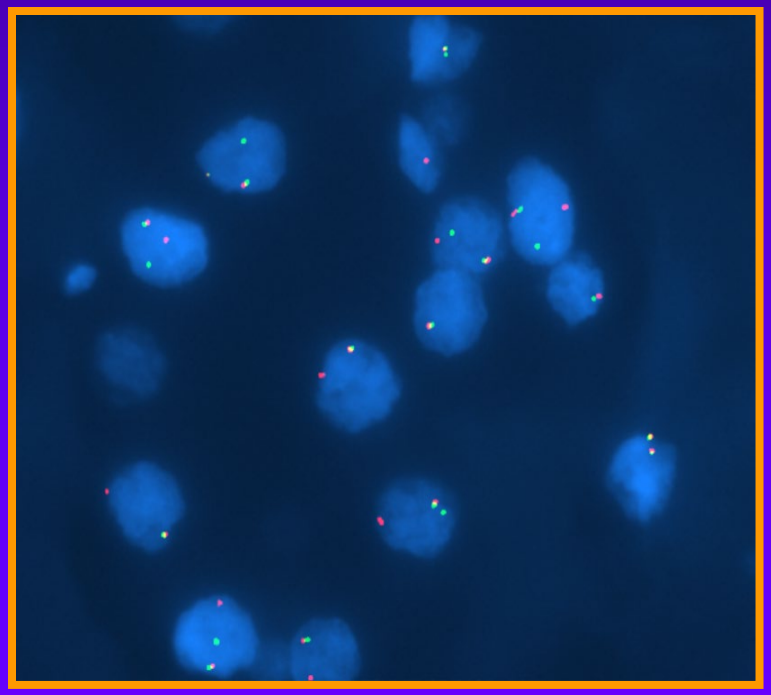
MAML2 *Among the most useful SG molecular probes for FNA*

A Reappraisal of the MECT1/MAML2 Translocation in Salivary Mucoepidermoid Carcinomas

Raja R. Seethala, MD, Sanja Dacic, MD, PhD, Kathleen Cieply, MS, Lindsey M. Kelly, BS, and Marina N. Nikiforova, MD

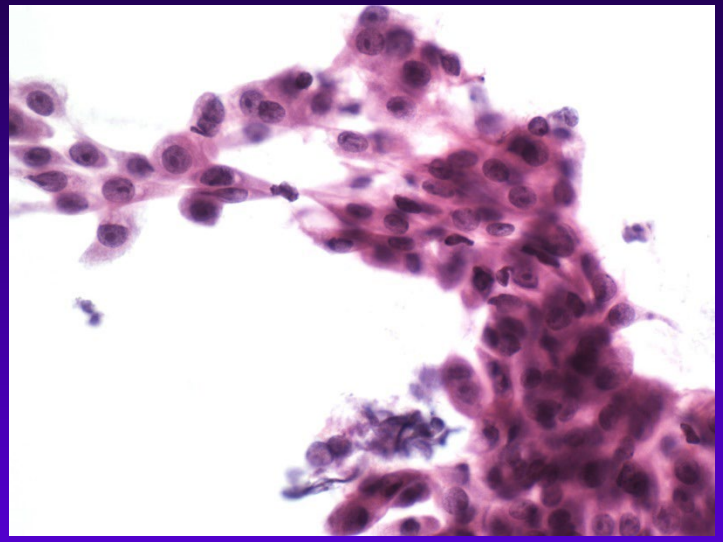
Cytogenetics:

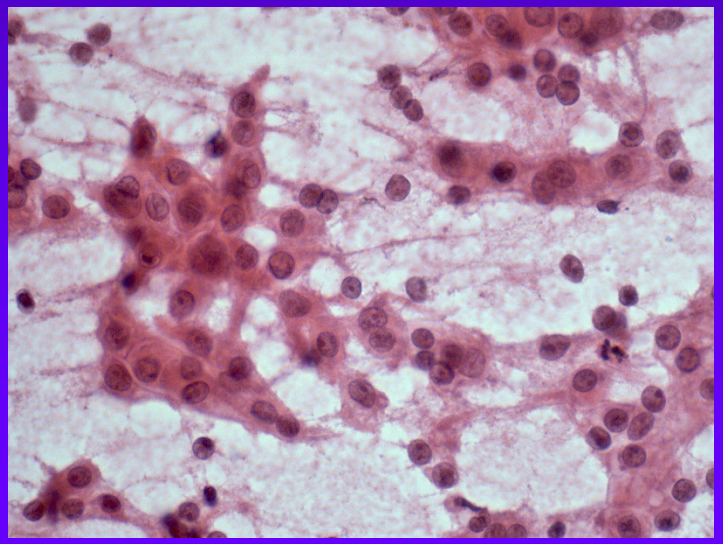
- t(11:19) translocation
- MECT1/MAML2
- FISH or NGS
- More common in low grade
- Often a better prognosis
  - >75%
  - LG-IG 75%, HG 32%

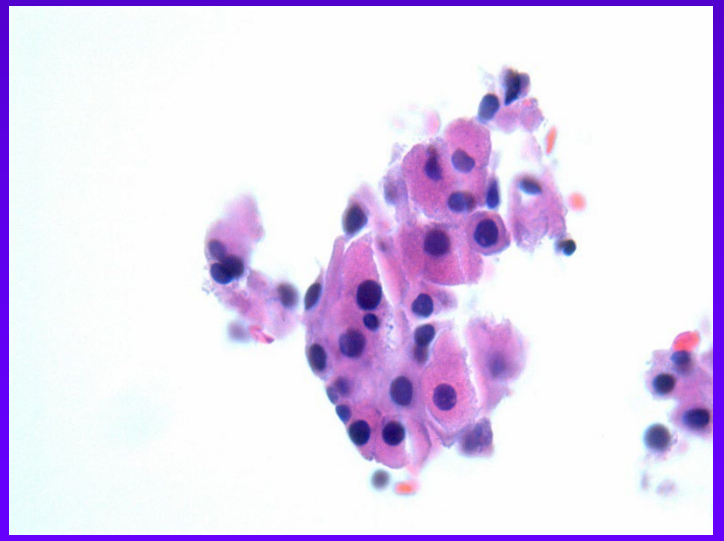


Mucoepidermoid Carcinoma:

3 FNA examples positive for MAML2 fusion

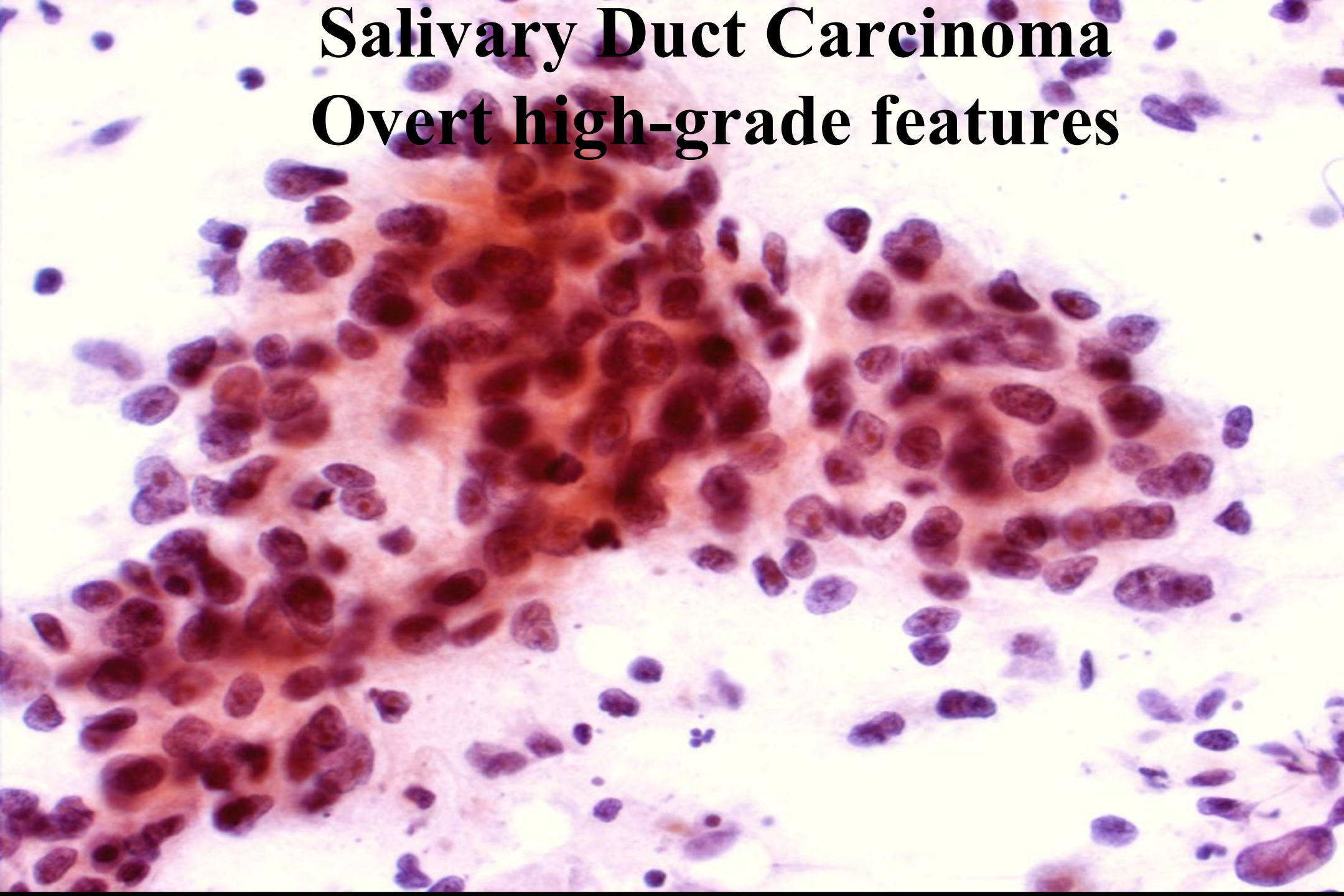






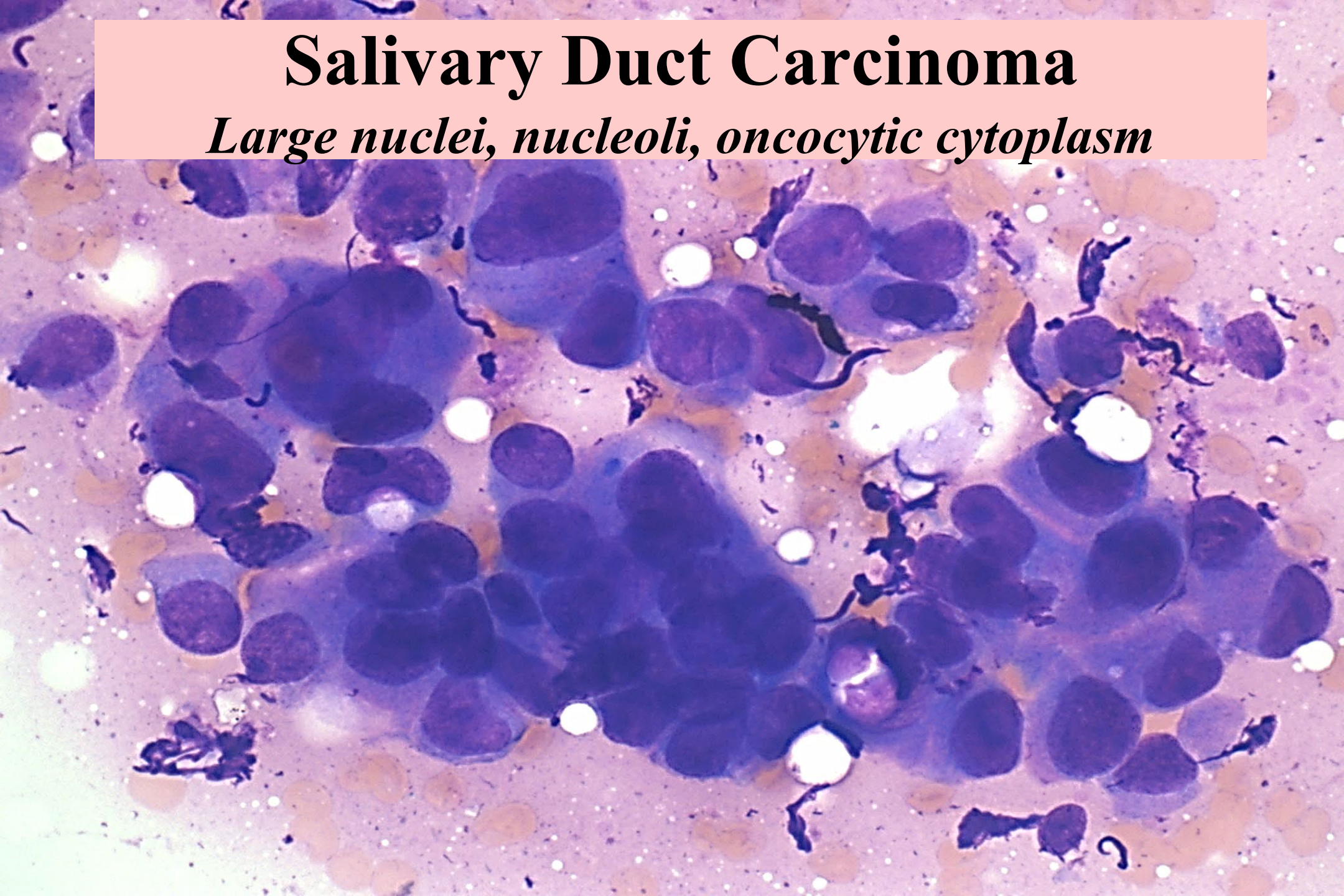
KEY POINTS

- **Mucoepidermoid Carcinoma Cytology**
  - **Background mucin**
  - **Epidermoid cells + goblet-type mucin cells**
  - **May be hypocellular**
  - **Most will be classified as SUSPICIOUS or SUMP**
  - **MAML2 fusions**


Salivary Duct Carcinoma
Overt high-grade features

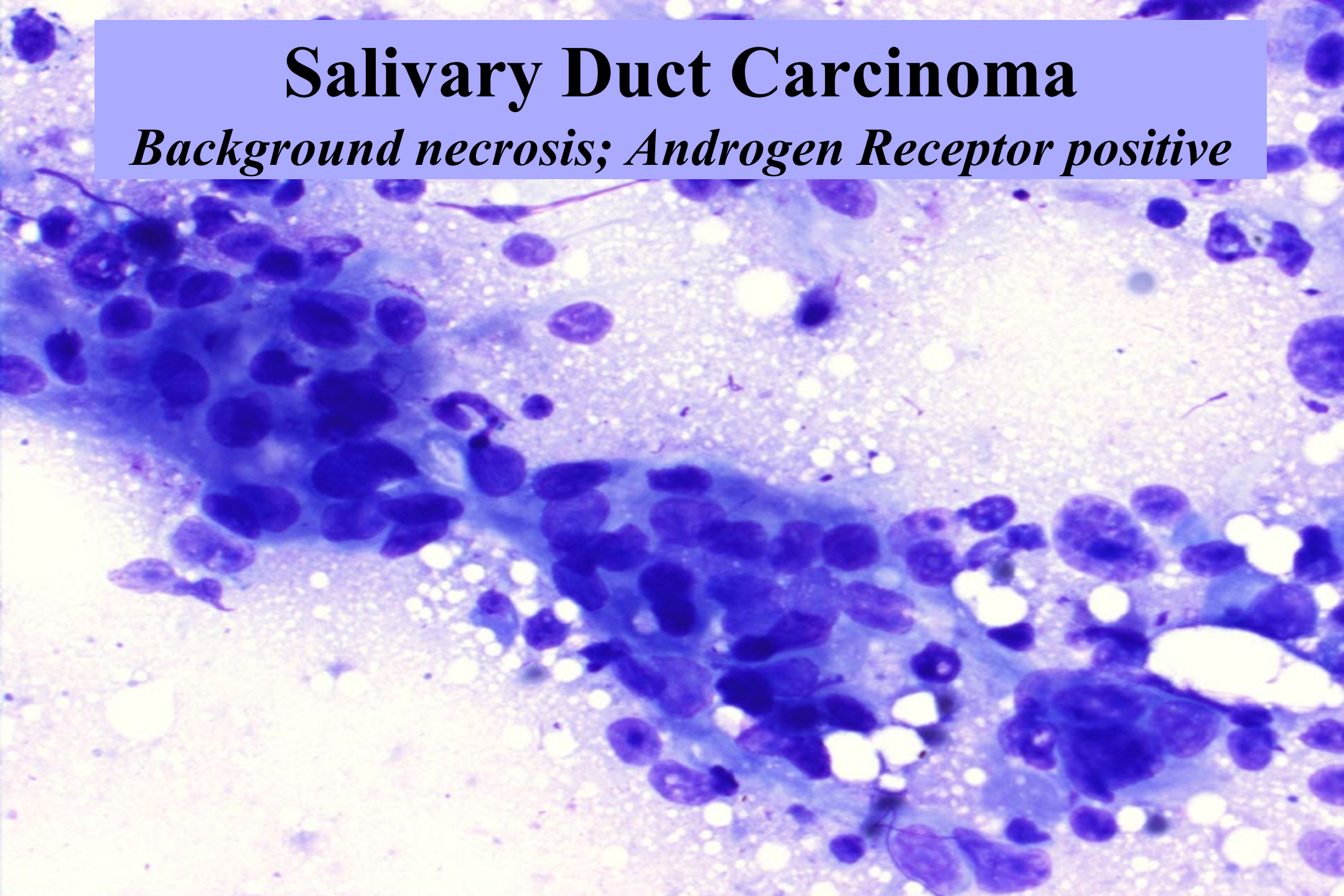
Salivary Duct Carcinoma

Large nuclei, nucleoli, oncocytic cytoplasm



Salivary Duct Carcinoma

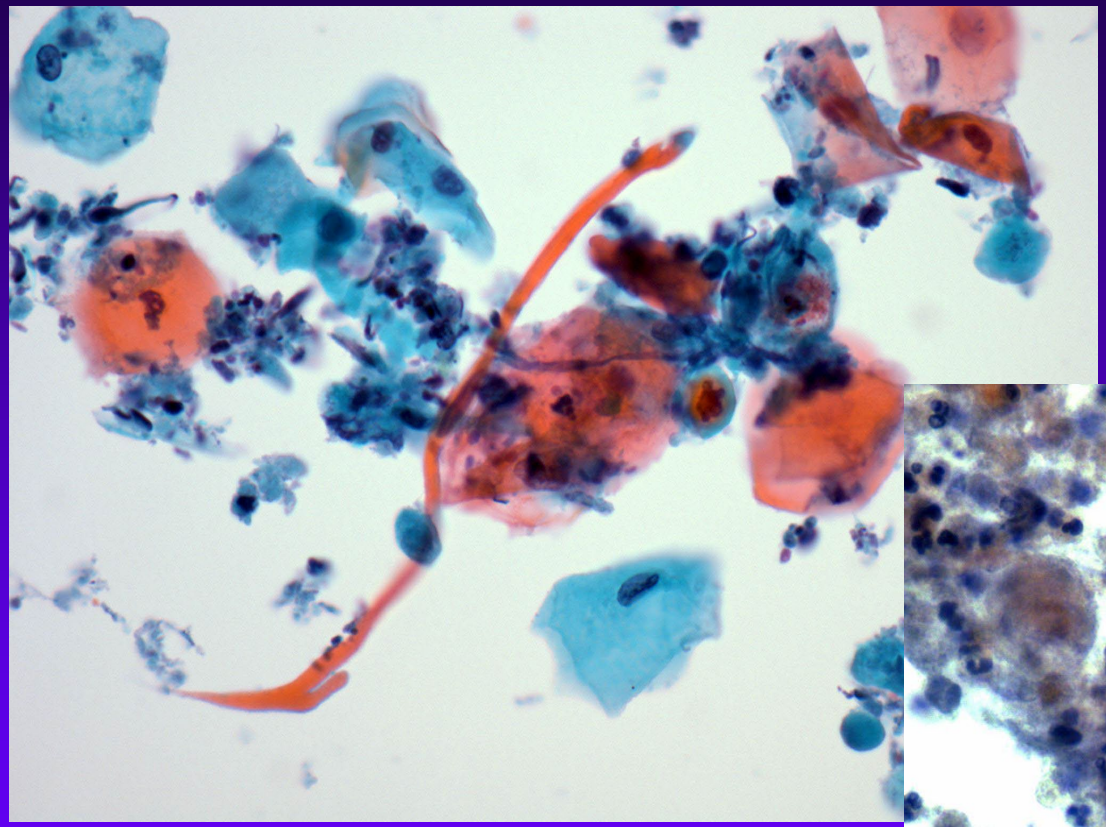
Background necrosis; Androgen Receptor positive



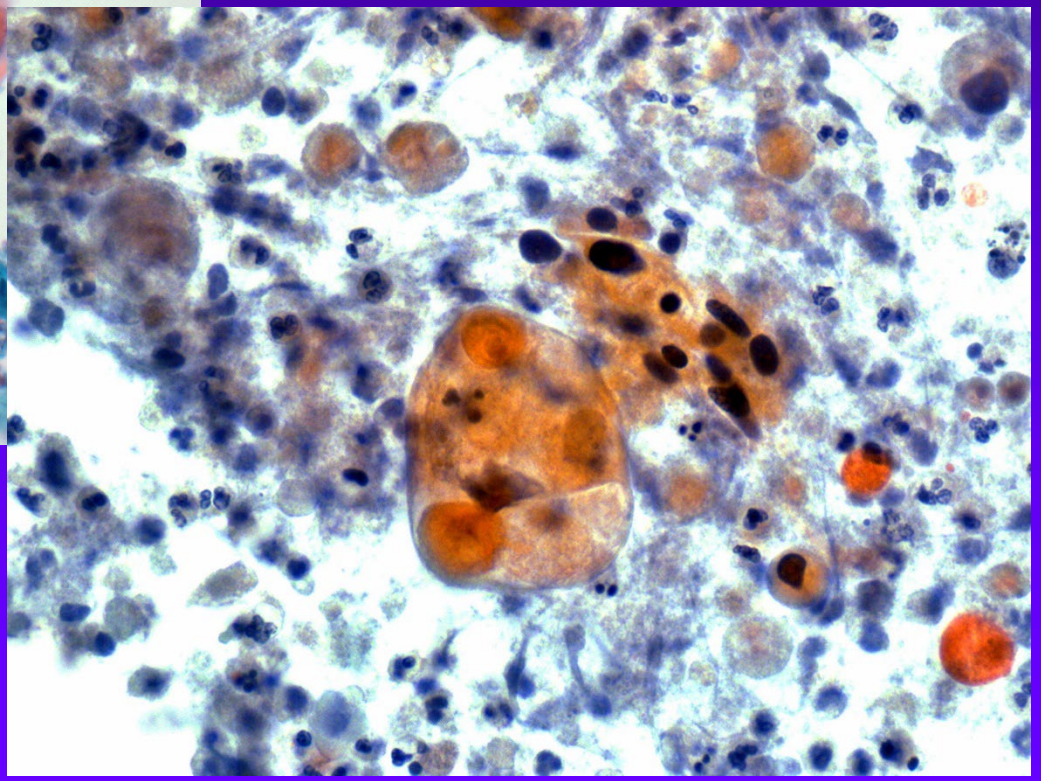
KEY POINTS

- **Salivary Duct Carcinoma Cytology**
  - **Large nuclei with distinct nucleolus**
  - **Moderate amounts of oncocytic cytoplasm**
  - **May have background necrosis**
  - **Most will be classified as Malignant/high-grade**
  - **Positive for Androgen recptor +/- HER2**

80 yo male with a 4 cm left parotid mass.



MALIGNANT:
Metastatic Skin Cancer




THANK YOU!