Oleksiy Tsybrovskyy, MD

VINCENT ACADEMY OF PATHOLOGY

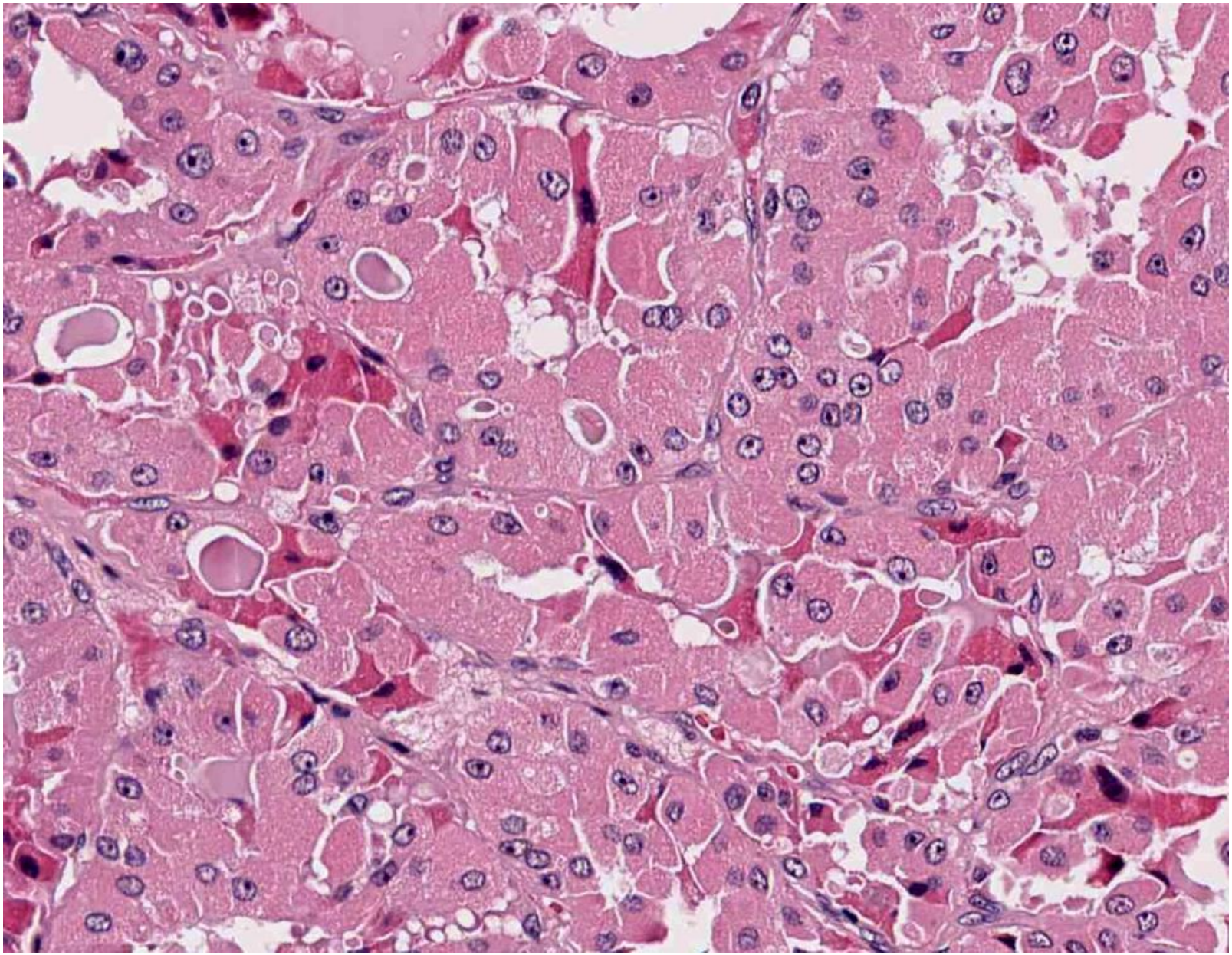
ONCOCYTIC THYROID LESIONS

BELIEFS, DOGMAS AND MYTHS

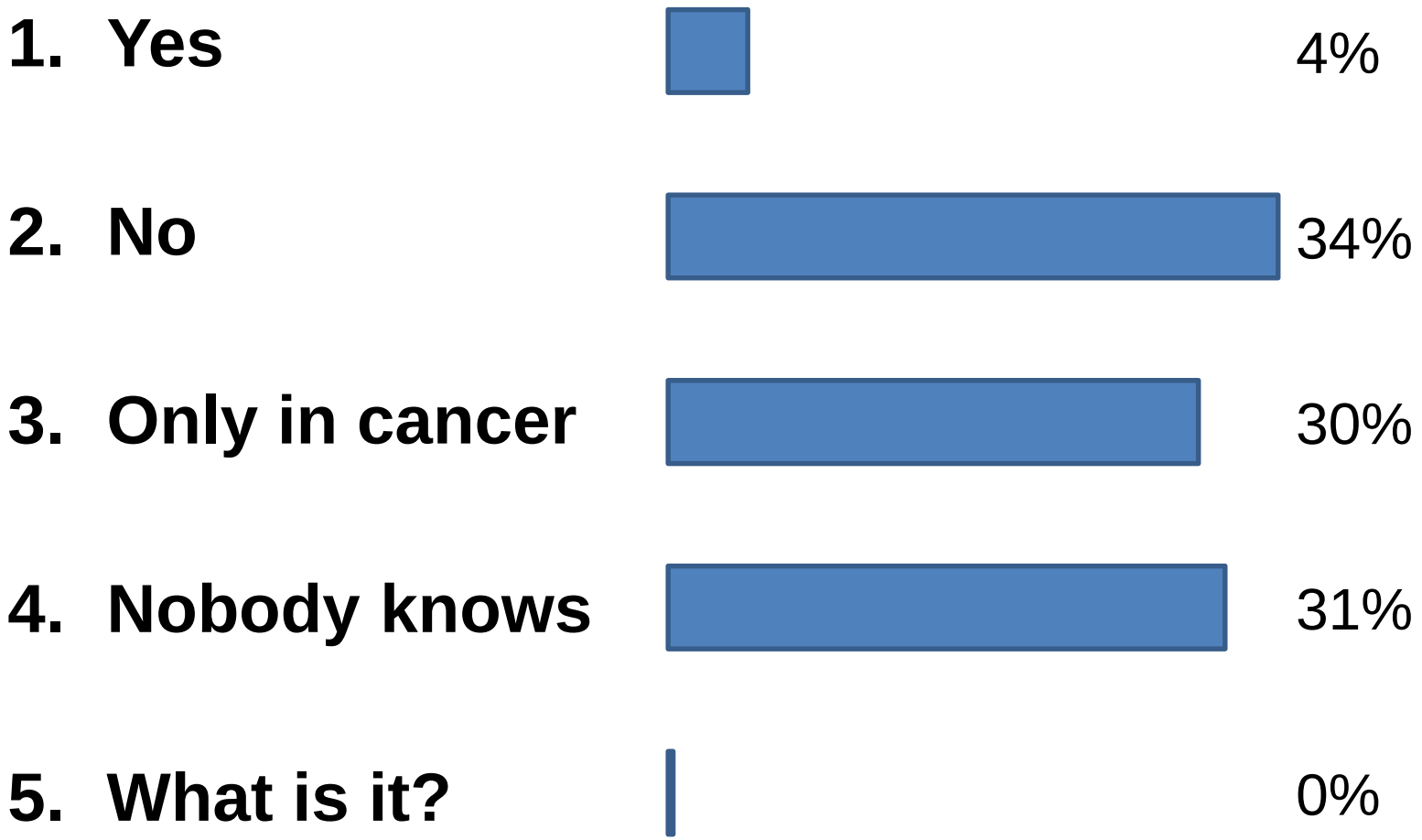
…and a piece of truth (maybe)

Disclosure

Nothing to disclose



Are oncocytes in the thyroid biologically more aggressive than non-oncocytes?



Prof. Manuel Sobrinho-Simões

"It is difficult to find a subject in thyroid pathology that has been more mistreated that that of Hürthle (oncocytic) cell tumors"

Int J Surg Path 13(1):29-35, 2005

First historic milestones

- Schaffer, 1897: “granular, swollen cells” in salivary glands
- Askanazy, 1898: “oxyphilic” cells in the thyroid

(Aus dem Pathologisch-anatomischen Institut der Universität Wien und dem Laboratorium der österreichischen Gesellschaft zur Erforschung und Bekämpfung der Krebskrankheit in Wien. [Vorstand: Prof. *R. Maresch*].)

Onkocyten und Geschwülste der Speicheldrüsen[1].

Von

H. Hamperl.

Mit 5 Abbildungen im Text.

(Eingegangen am 8. Juli 1931.)

- Jaffé, 1932: “oncocytoma” (adenolymphoma) of the parotid gland

When the things go wrong…

USA, 1950s:

- “Hürthle-cells” (misnomer) instead of oncocytes
- “Hürthle cell adenoma” is obsolete and undesirable. It is recommended that the term be replaced by “Hürthle cell carcinoma or adenocarcinoma” (Manual of Tumor Nomenclature and Coding, American Cancer Society, 1951)
- Several impressive US series (due to large numbers of “Hürthle cell carcinomas”) followed
- It took about 20-30 years to finally get rid of this “malignancy” mythos

Good old Europe

Hamperl: series of papers on “oncocytes” and “oncocytomas” between 1931 and 1961 in various organs, including thyroid:

- Most oncocytomas are benign (criticism on the “malignancy mania” in the US)
- Loss of normal function (hormonally inactive)
- Association with patient’s age (a kind of “cellular aging”)
- “Oncocytic metaplasia represents some form of mild cellular degeneration or regression”

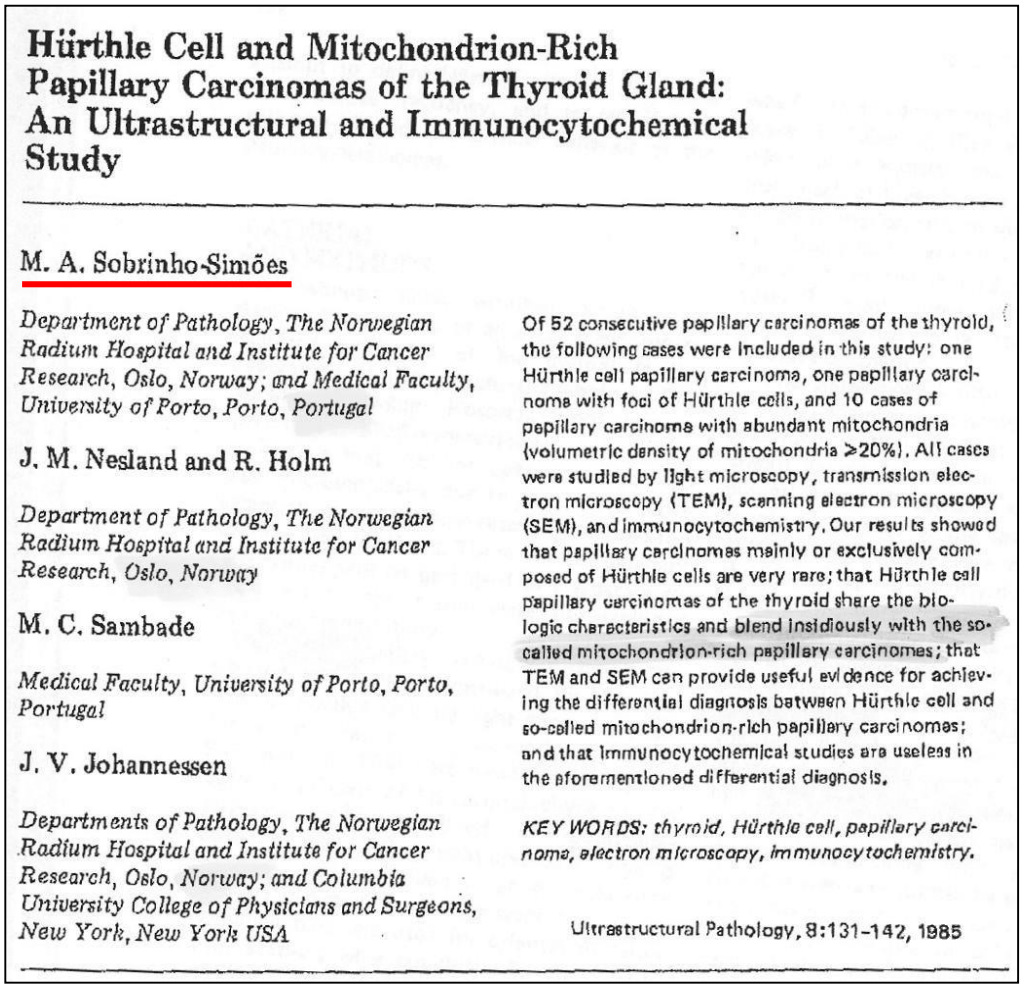
Tremblay & Pearse, 1959-1960:

- histochemical demonstration of highly increased mitochondrial enzyme activity in oncocytes
- => “*increased formation of biochemically defective mitochondria due to an increased demand*”
- “mitochondrion-rich” cells in goiter (similar to oncocytes)

Ultrastructural studies in 1960-1970s

- Abundance of (often structurally abnormal) mitochondria in oncocytes universally confirmed in all organs and sites
- Feldman, 1972:
  - Closely packed mitochondria obscure other organelles
  - Apical parts of cytoplasm contain RER and lysosomes
- Böcker, 1978:
  - Four cell types based on ultrastructure:
    - Ergastoplasm-rich cells
    - Clear cell
    - Oxiphil cells (typical oncocytes)
    - Mitochondrion-rich cells (accumulation of mitochondria in basal parts of the cytoplasm)

1980s: Along came ~~Polly…?~~ Manuel



Hürthle Cell and Mitochondrion-Rich Papillary Carcinomas of the Thyroid Gland: An Ultrastructural and Immunocytochemical Study

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Departments of Pathology, The Norwegian Radium Hospital and Institute for Cancer Research, Oslo, Norway; and Columbia University College of Physicians and Surgeons, New York, New York USA

Of 52 consecutive papillary carcinomas of the thyroid, the following cases were included in this study: one Hürthle cell papillary carcinoma, one papillary carcinoma with foci of Hürthle cells, and 10 cases of papillary carcinoma with abundant mitochondria (volumetric density of mitochondria ≥20%). All cases were studied by light microscopy, transmission electron microscopy (TEM), scanning electron microscopy (SEM), and immunocytochemistry. Our results showed that papillary carcinomas mainly or exclusively composed of Hürthle cells are very rare; that Hürthle cell papillary carcinomas of the thyroid share the biologic characteristics and blend insidiously with the so-called mitochondrion-rich papillary carcinomas; that TEM and SEM can provide useful evidence for achieving the differential diagnosis between Hürthle cell and so-called mitochondrion-rich papillary carcinomas; and that immunocytochemical studies are useless in the aforementioned differential diagnosis.

KEY WORDS: thyroid, Hürthle cell, papillary carcinoma, electron microscopy, immunocytochemistry.

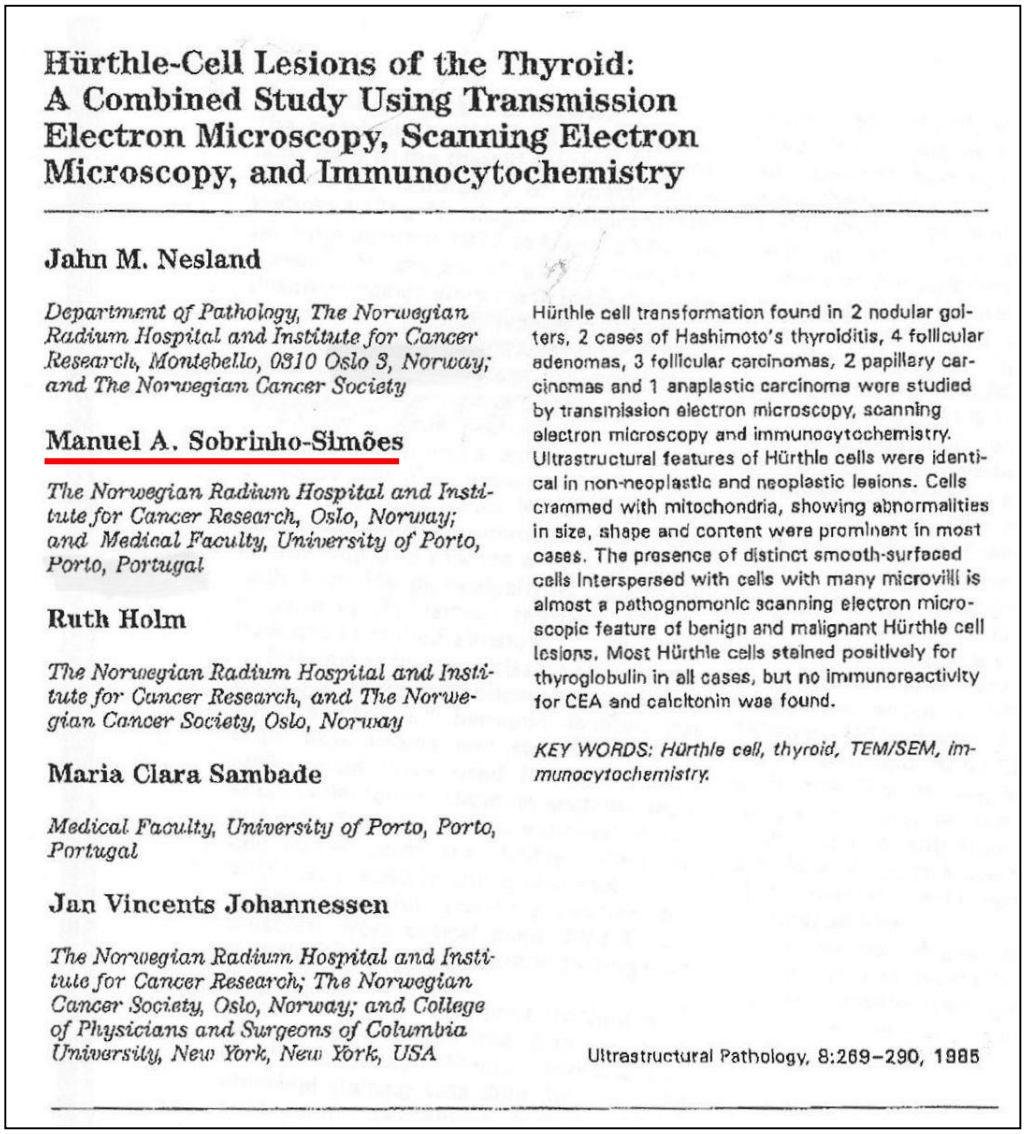
Ultrastructural Pathology, 8:131–142, 1985

52 PTCs studied, of them:

- 2 overt Hürthle-cell (by light microscopy)
- 10 "eosinophilic" = "tall cell" = "mitochondrion-rich" on TEM

"Apart from the prominence of some mitochondrial and cell surface abnormalities usually found in Hürthle cell tumors, there are no clear-cut differences between mitochondrion-rich and "true" Hürthle cell carcinomas…"

1980s: Pivotal studies



Hürthle-Cell Lesions of the Thyroid: A Combined Study Using Transmission Electron Microscopy, Scanning Electron Microscopy, and Immunocytochemistry

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Maria Clara Sambade

Medical Faculty, University of Porto, Porto, Portugal

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The Norwegian Radium Hospital and Institute for Cancer Research; The Norwegian Cancer Society, Oslo, Norway; and College of Physicians and Surgeons of Columbia University, New York, New York, USA

Hürthle cell transformation found in 2 nodular goiters, 2 cases of Hashimoto's thyroiditis, 4 follicular adenomas, 3 follicular carcinomas, 2 papillary carcinomas and 1 anaplastic carcinoma were studied by transmission electron microscopy, scanning electron microscopy and immunocytochemistry. Ultrastructural features of Hürthle cells were identical in non-neoplastic and neoplastic lesions. Cells crammed with mitochondria, showing abnormalities in size, shape and content were prominent in most cases. The presence of distinct smooth-surfaced cells interspersed with cells with many microvilli is almost a pathognomonic scanning electron microscopic feature of benign and malignant Hürthle cell lesions. Most Hürthle cells stained positively for thyroglobulin in all cases, but no immunoreactivity for CEA and calcitonin was found.

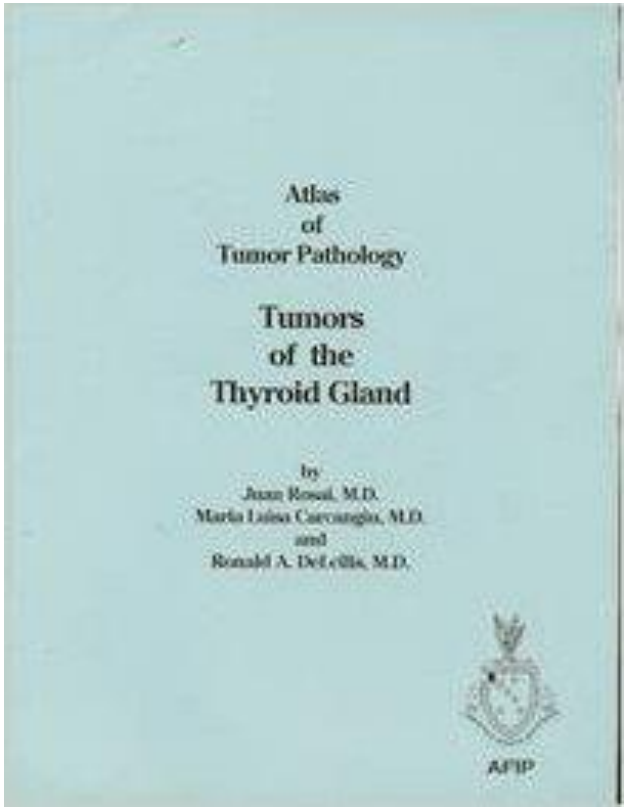
KEY WORDS: Hürthle cell, thyroid, TEM/SEM, immunocytochemistry.

Ultrastructural Pathology, 8:269–290, 1985

"Hürthle cell transformation is not a "black or white" phenomenon, but a stepwise process leading to more abundant mitochondria."

"In our opinion and for practical purposes, we think that the light microscopic definition of Hürthle cells is sufficient, regardless of the degree of ultrastructural abnormality.
...by light microscopy, the differential diagnosis [from pseudo-oncocytes] *can almost always be made."*

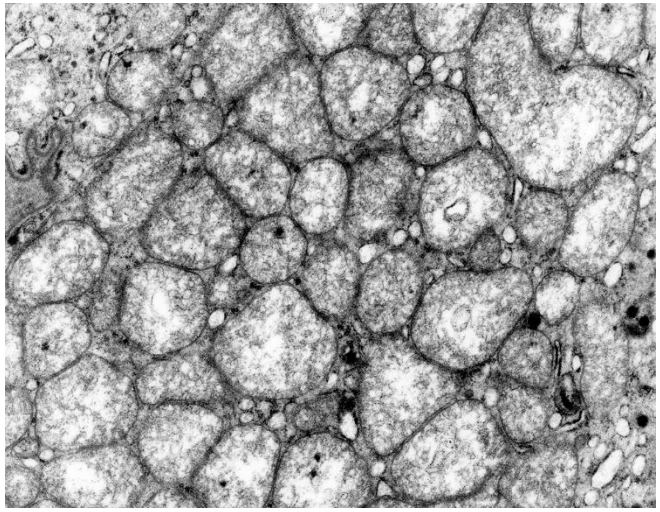
Juan Rosai, 1992:

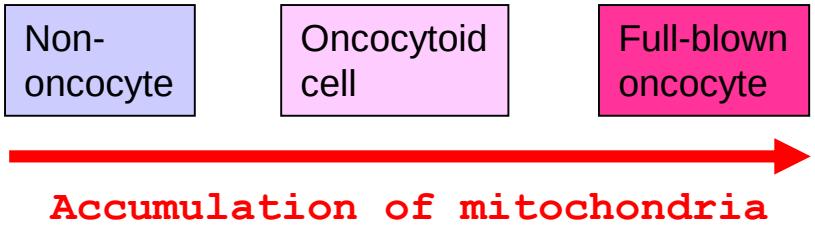


- Oncocytes can be reliably recognized on HE alone
- Use of IHC mitochondrial markers is not necessary

Concept of oncocytic change since 1990s:

- Extremely mitochondrion-rich
- Spectrum of changes:





- Oncocytic change is easy to recognize on H&E alone
- Mitochondrial accumulation = oncocytic change
- Mitochondrial accumulation is paralleled by increasing cytoplasmic oxiphilia
- It is all about mitochondria (apical displacement of other organelles ignored/forgotten

Immunohistochemistry ?

Applied Immunohistochemistry 2(4): 261–267, 1994

Immunocytochemical Identification of Oxyphilic Mitochondrion-Rich Cells

Mauro Papotti, M.D., Patrizia Gugliotta, B.Sc.,
Giuseppe Forte, M.D., and Gianni Bussolati, M.D., F.R.C.Path.

Few cases, especially benign:

- 3 goiters
- 3 Hashimoto
- 2 adenomas

"… the extent of oxyphilic transformation in some cases might not be so prominent, and the typical features of large granular eosinophilic cytoplasm may be missing. This is true especially in poorly differentiated oxyphilic tumors, as observed in some Hürthle cell carcinomas…"

"The marker tested here can be useful to reach more definite and reproducible criteria for the definition of mitochondrion-rich oxyphilic cells."

Study sample

928 samples

<u>470 follicular tumours:</u>

- 273 „cold“ adenomas
- 3 „toxic“ adenomas
- 194 carcinomas

<u>Controls:</u>

- 8 papillary cancers
- 1 medullary cancer

<u>458 non-neoplastic tissues:</u>

- 163 normal thyroids
- 295 diseased thydoids (Hashimoto, nodular goiter, Graves‘ disease. etc.)

Methods

- Tissue microarrays
- Mitochondrial markers: clone 113-1 and prohibitin
- DAKO polymer detection kit (biotin-free)
- Validation by electron microscopy in 15 cases

  (including volumetric density measurements)

Normal thyroid

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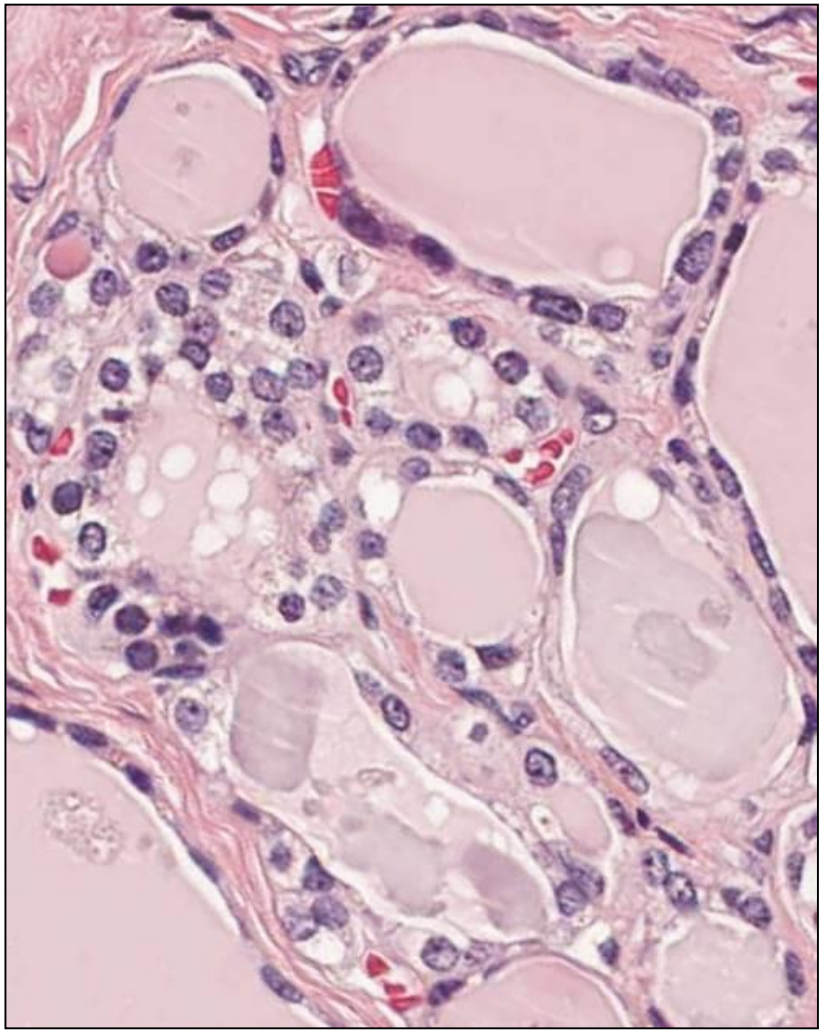
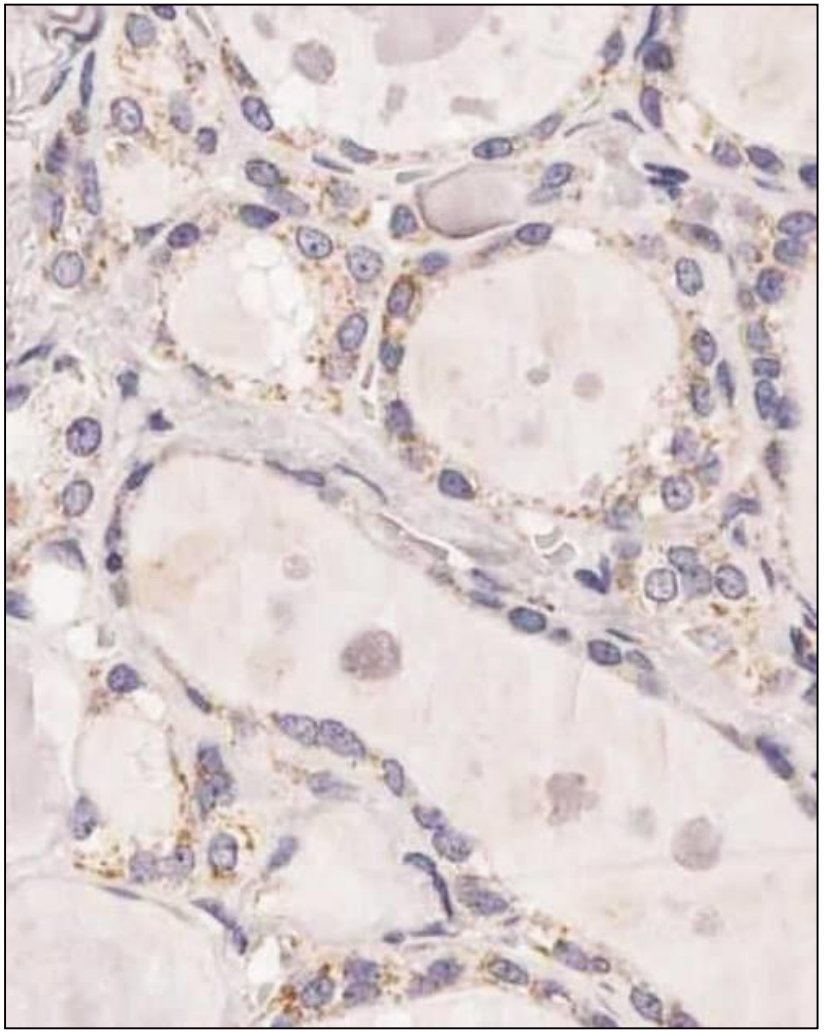
H&E



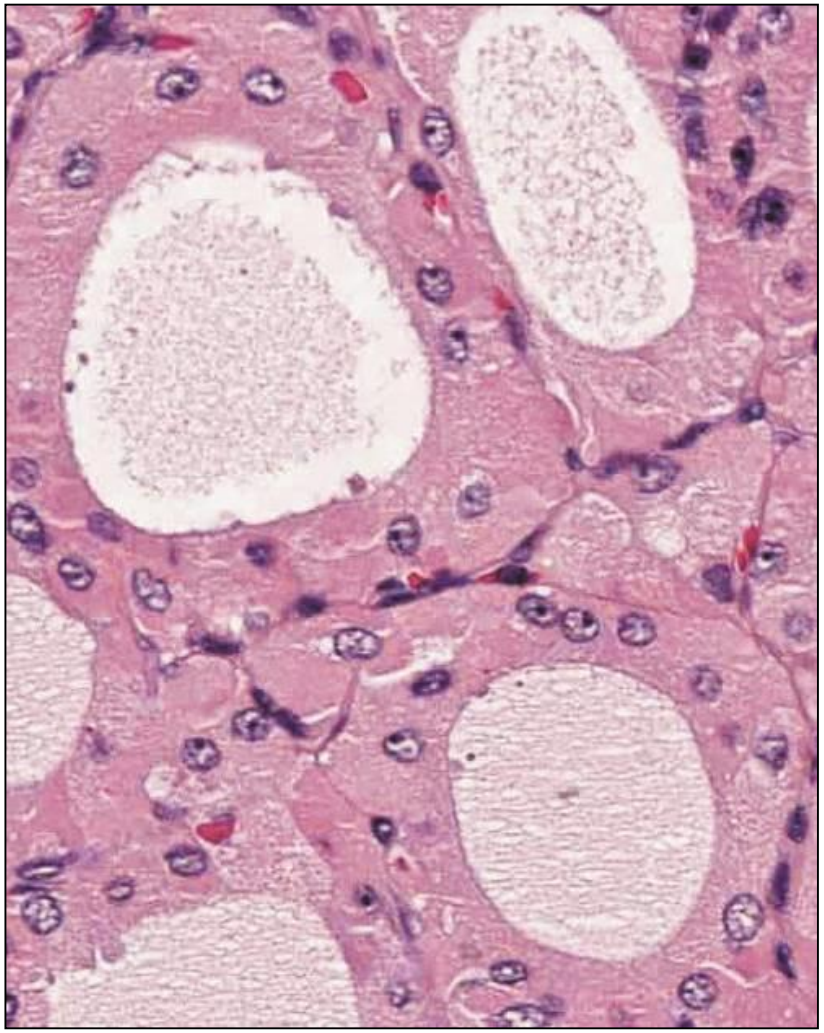
Mitochondria



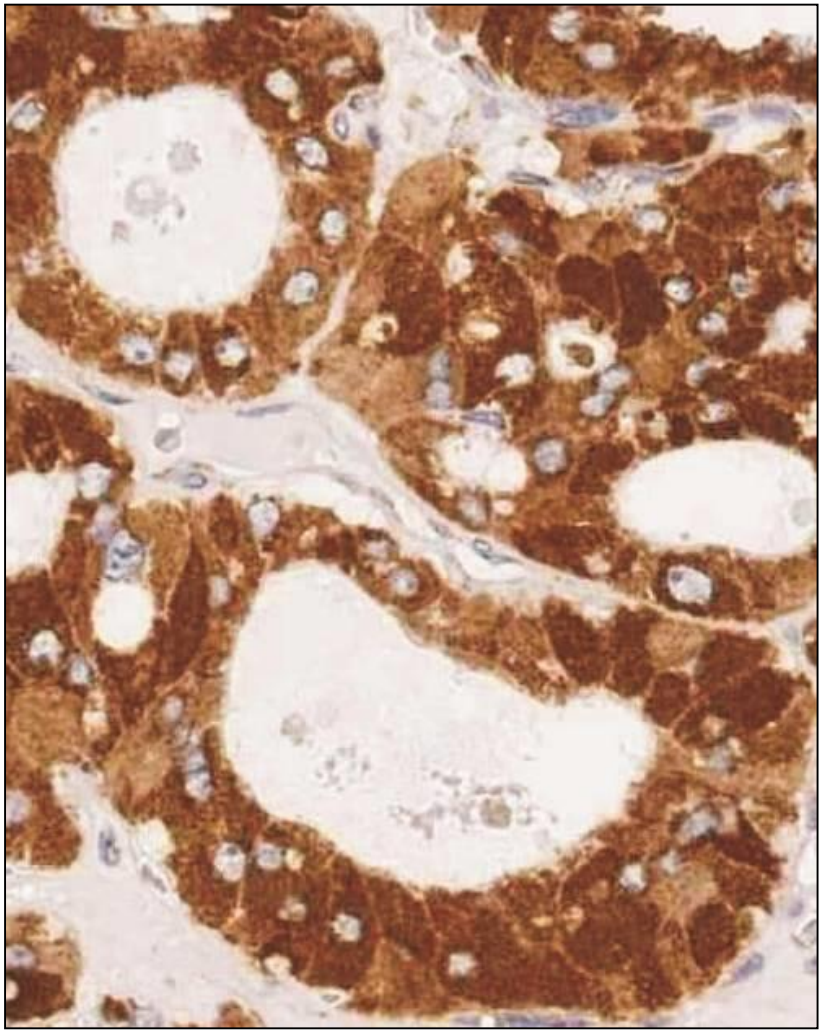
Classic oncocytes

VINCENT ACADEMY OF PATHOLOGY

H&E



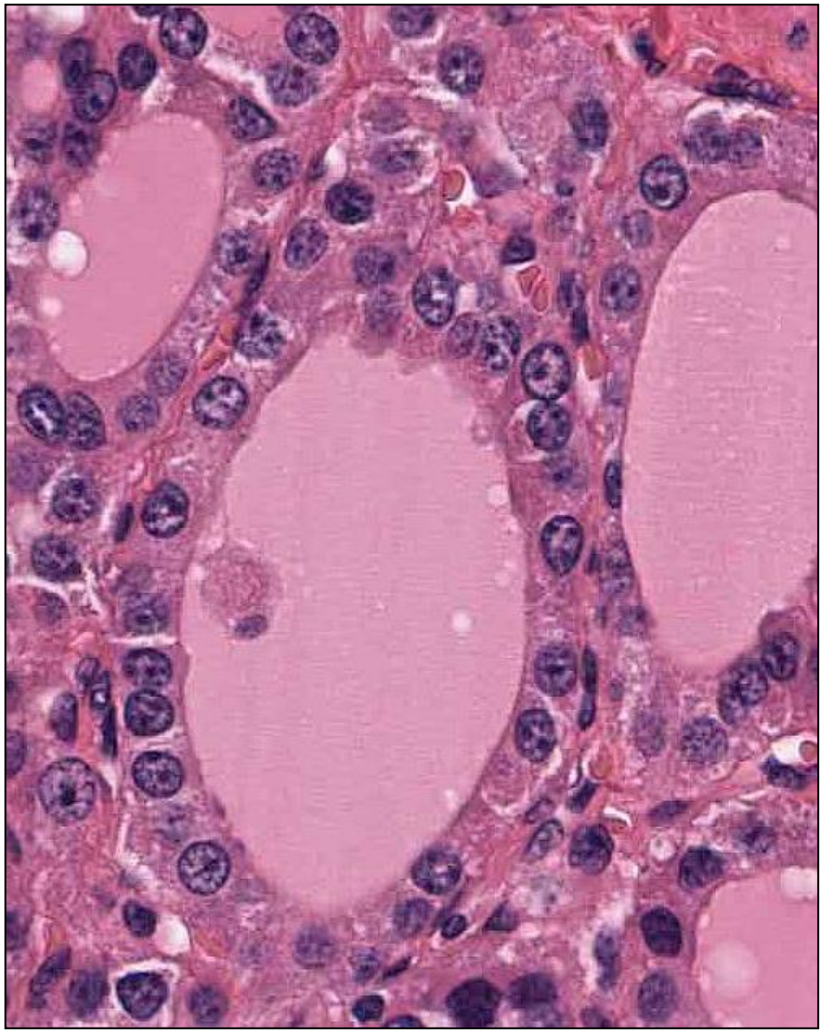
Mitochondria



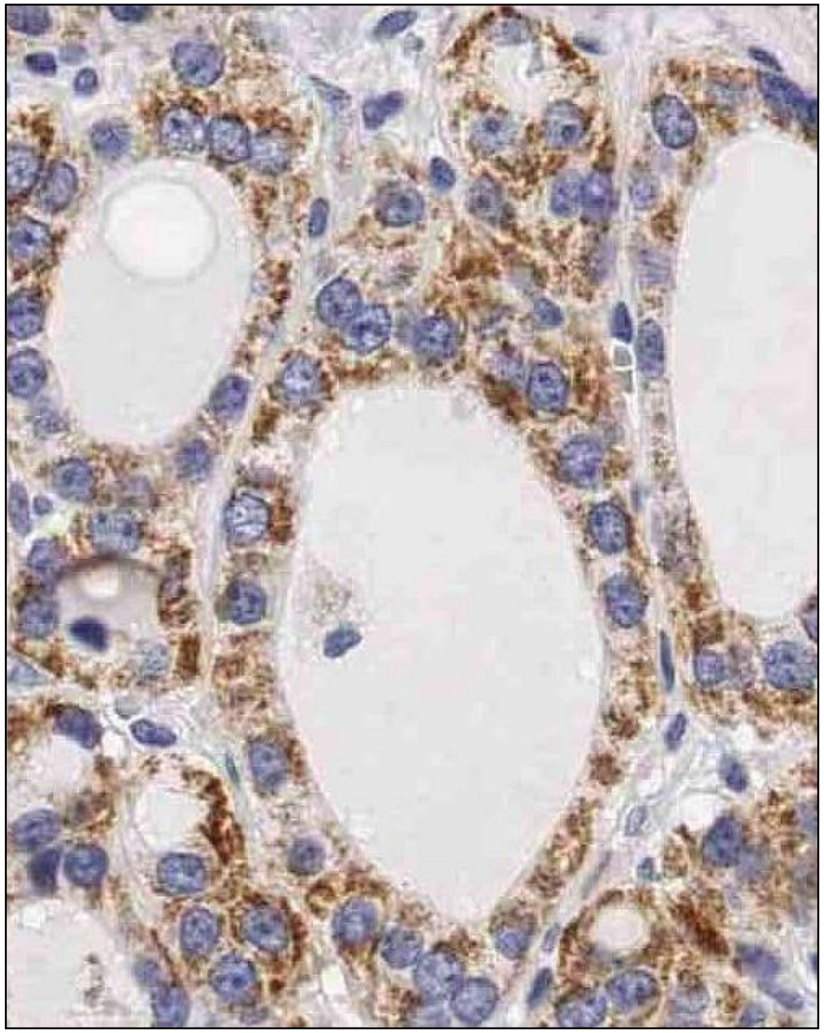
Obviously non-oncocytes

VINCENT ACADEMY OF PATHOLOGY

H&E



Mitochondria



Surprise no. 1: how many mitochondria?

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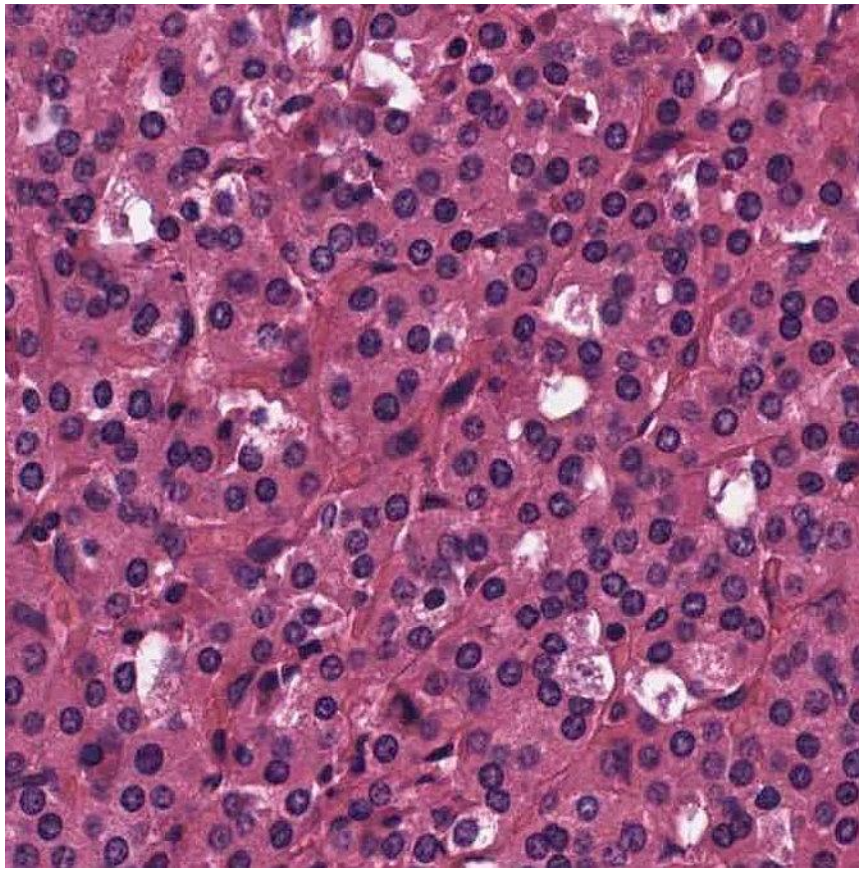
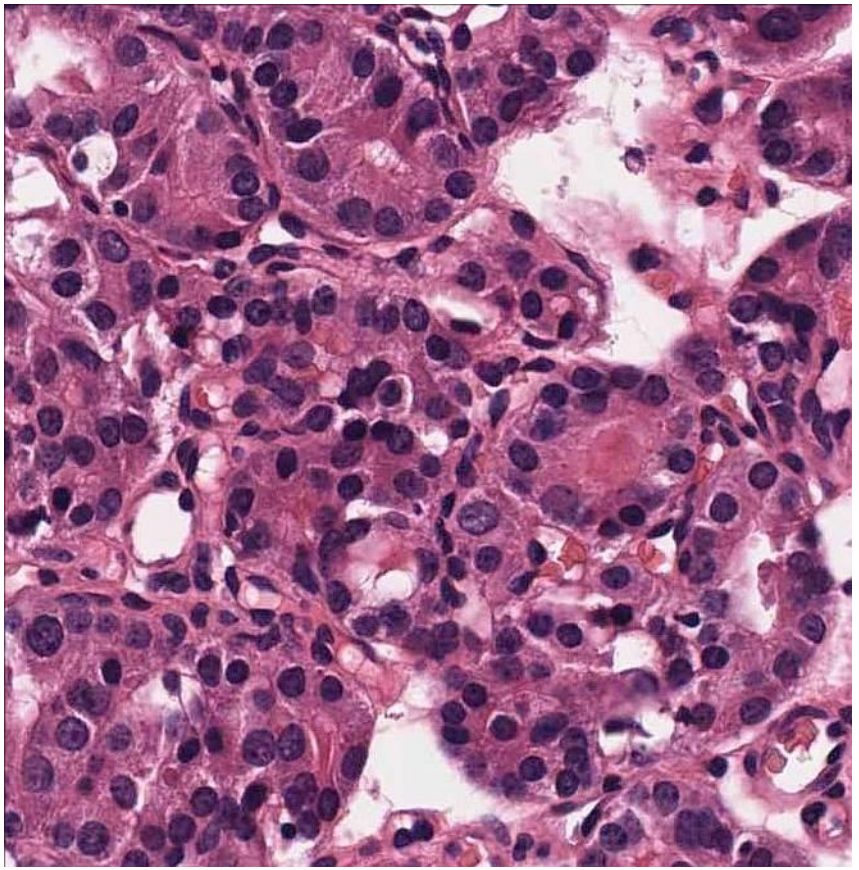
Tumor A



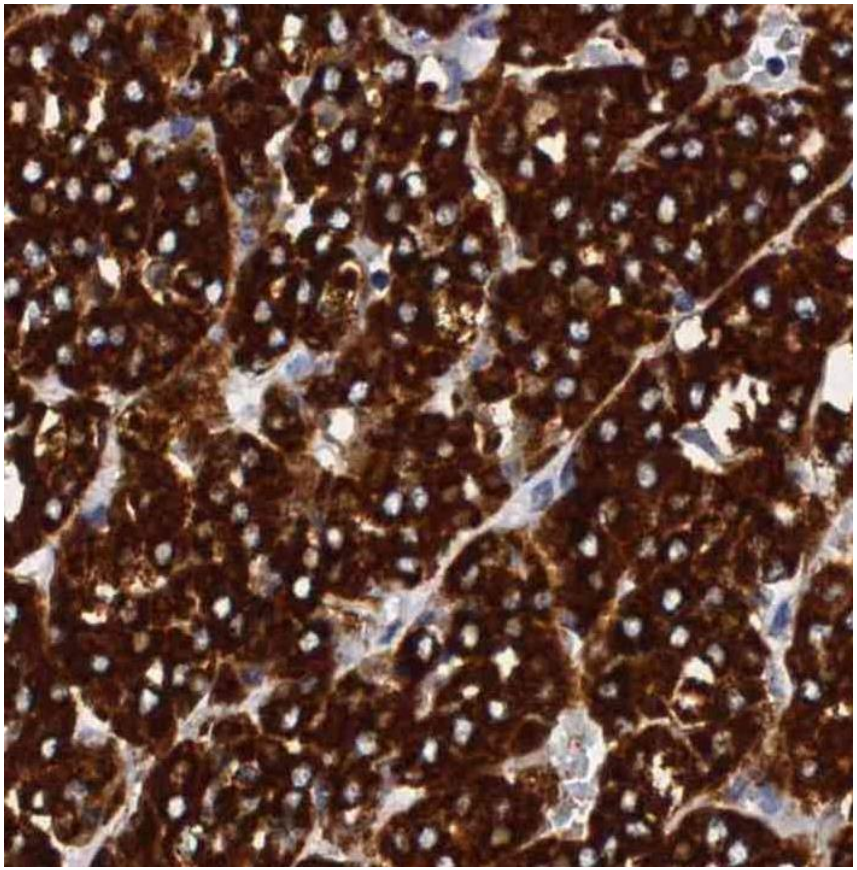
Tumor B



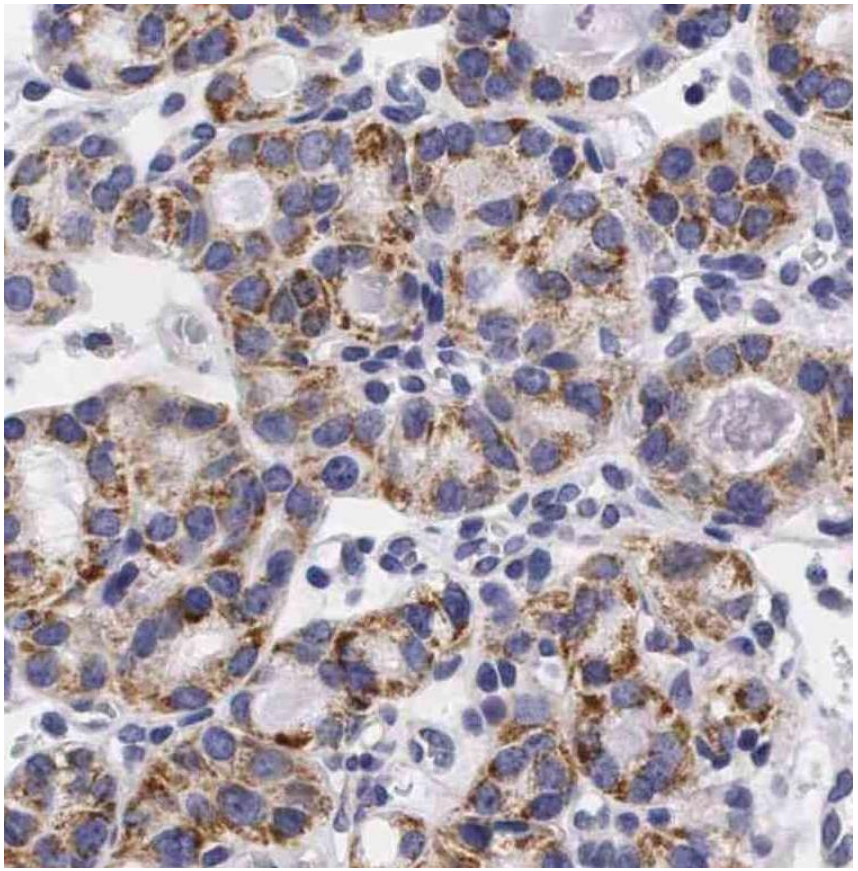
Mitochondrial immunostain

VINCENT ACADEMY OF PATHOLOGY

Tumor A



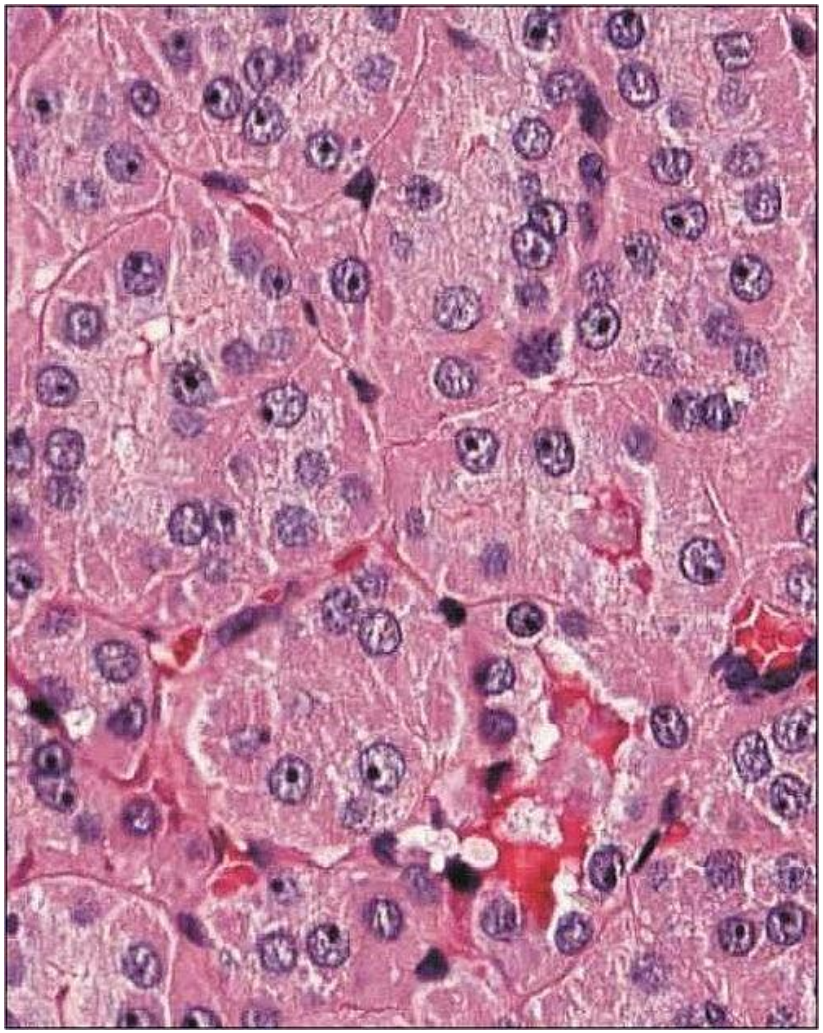
Tumor B



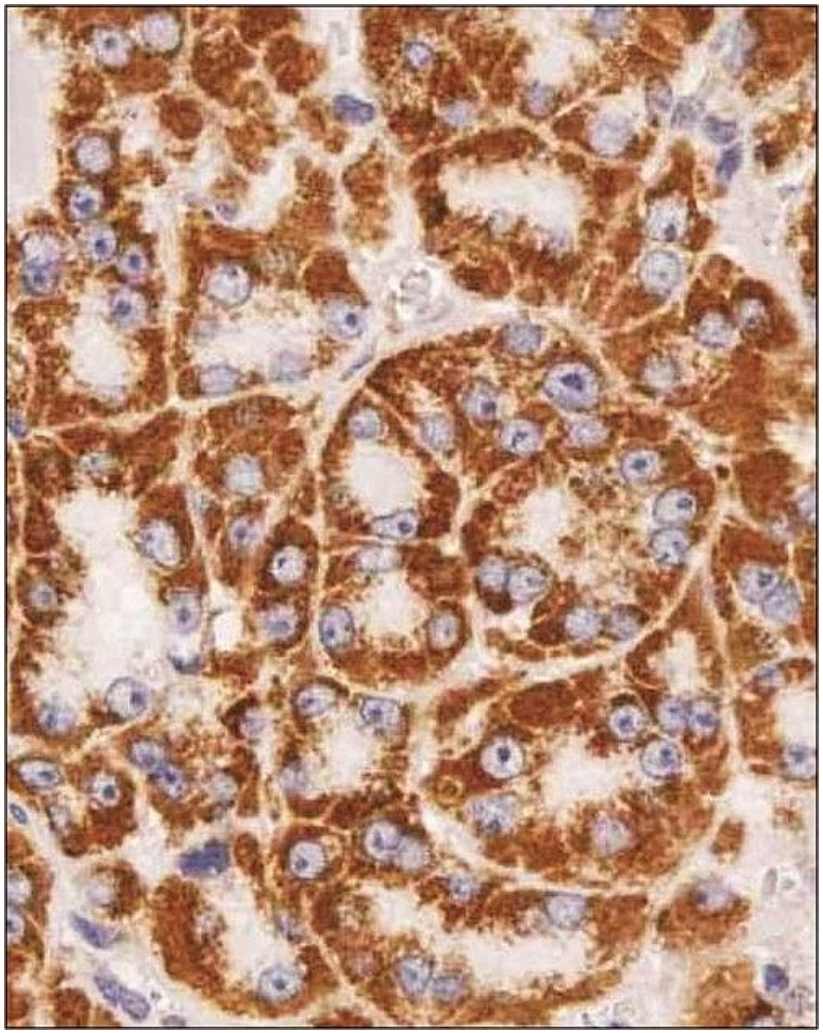
Surprise no. 2: are these oncocytes???

VINCENT ACADEMY OF PATHOLOGY

H&E



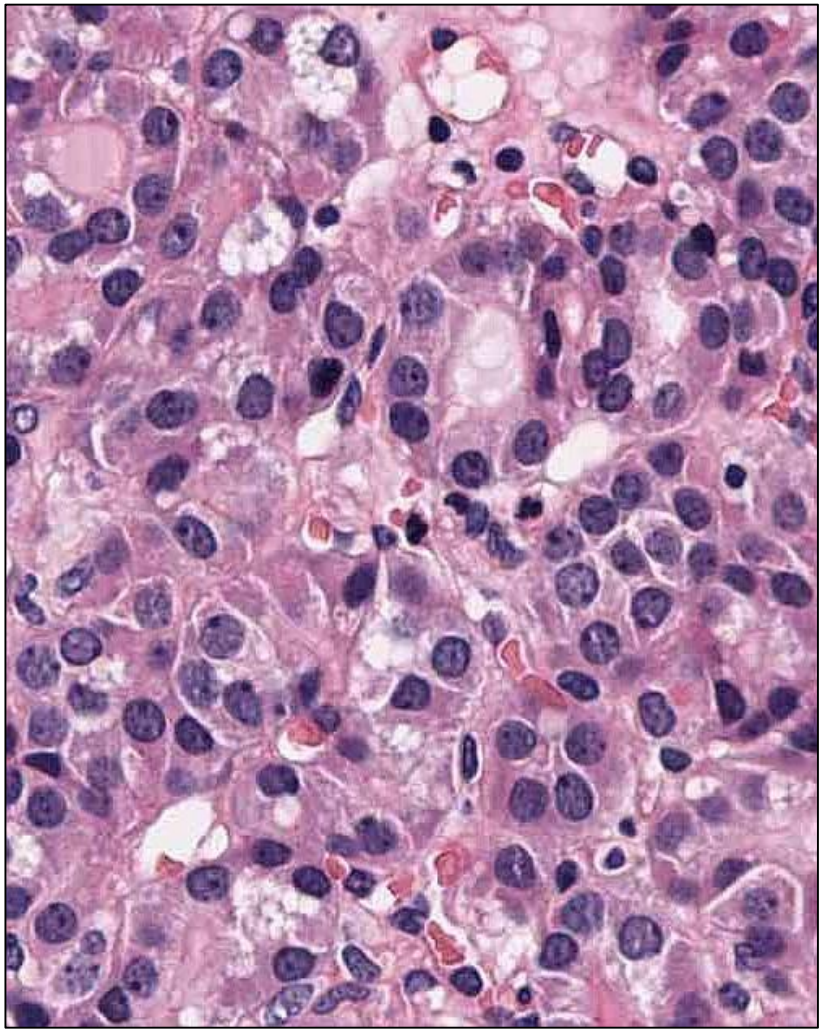
Mitochondria



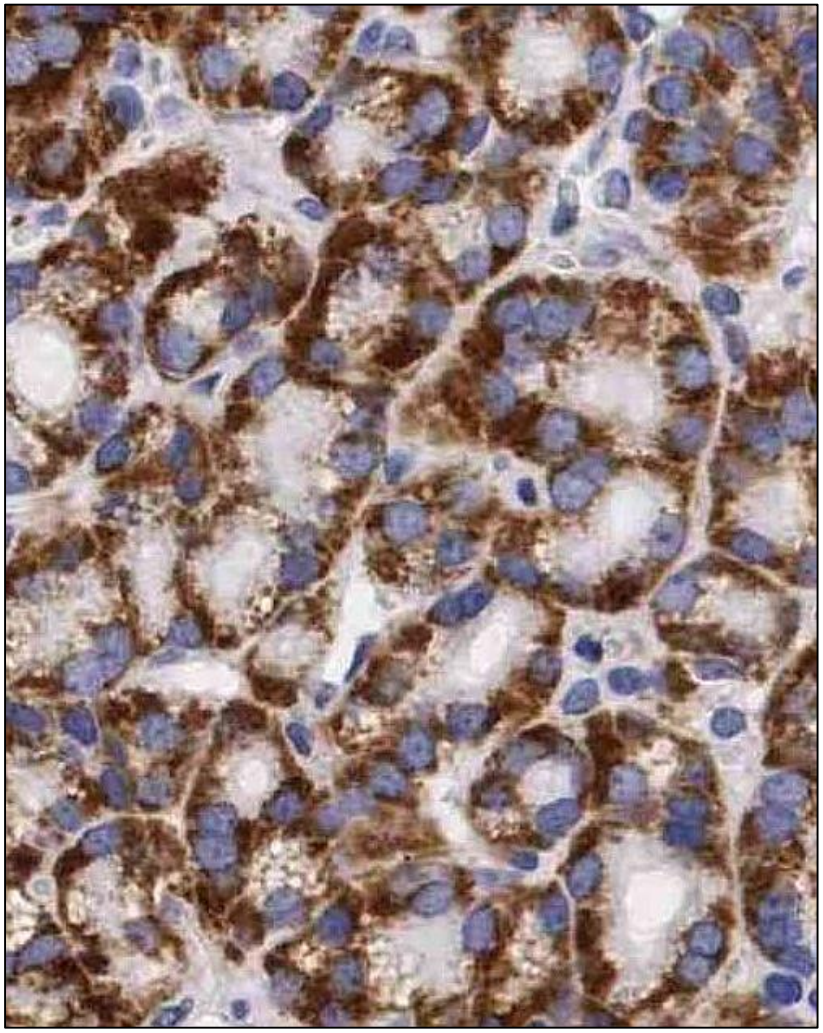
Or these? How to quantify the mitochondria here?

VINCENT ACADEMY OF PATHOLOGY

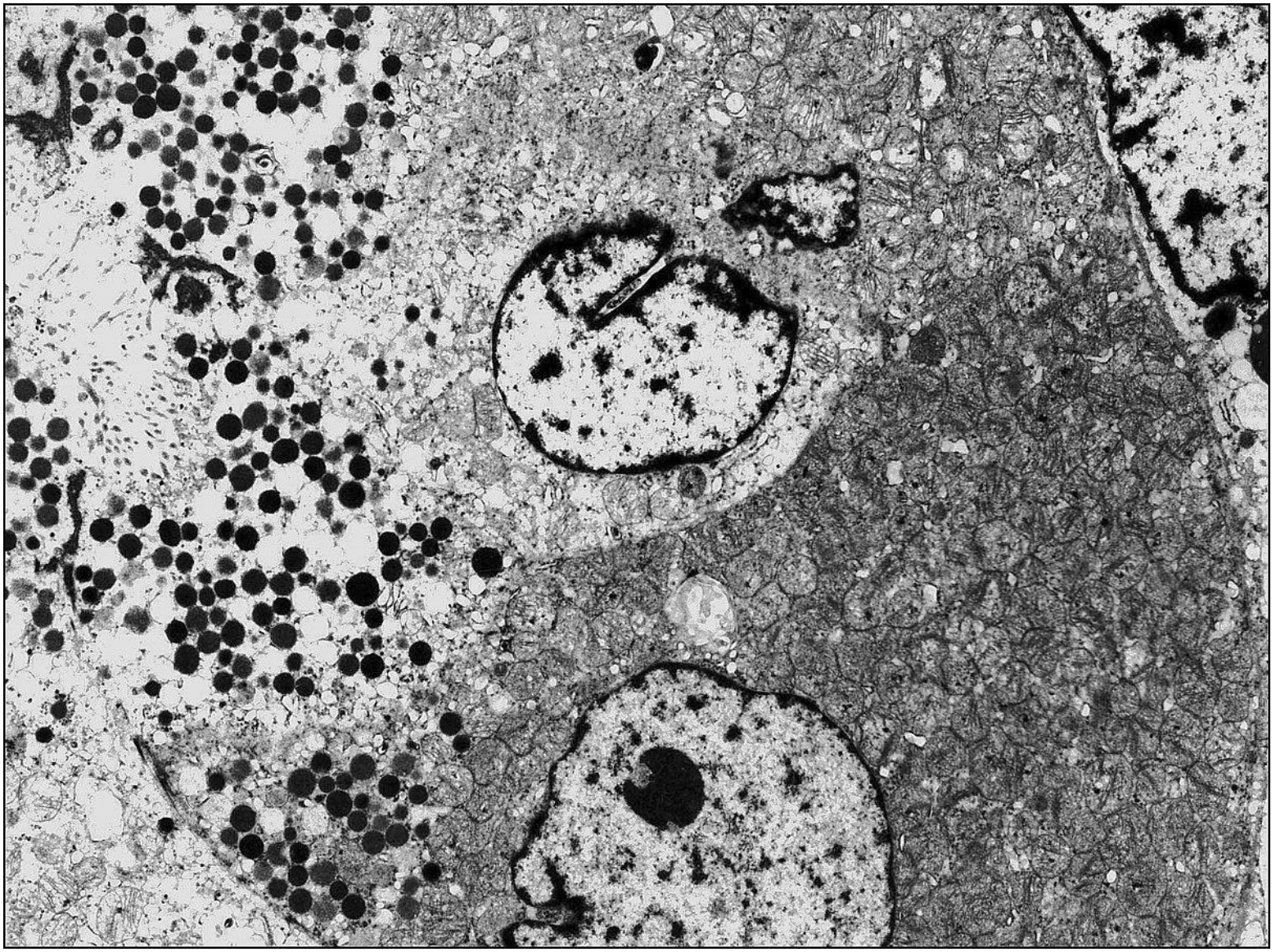
H&E



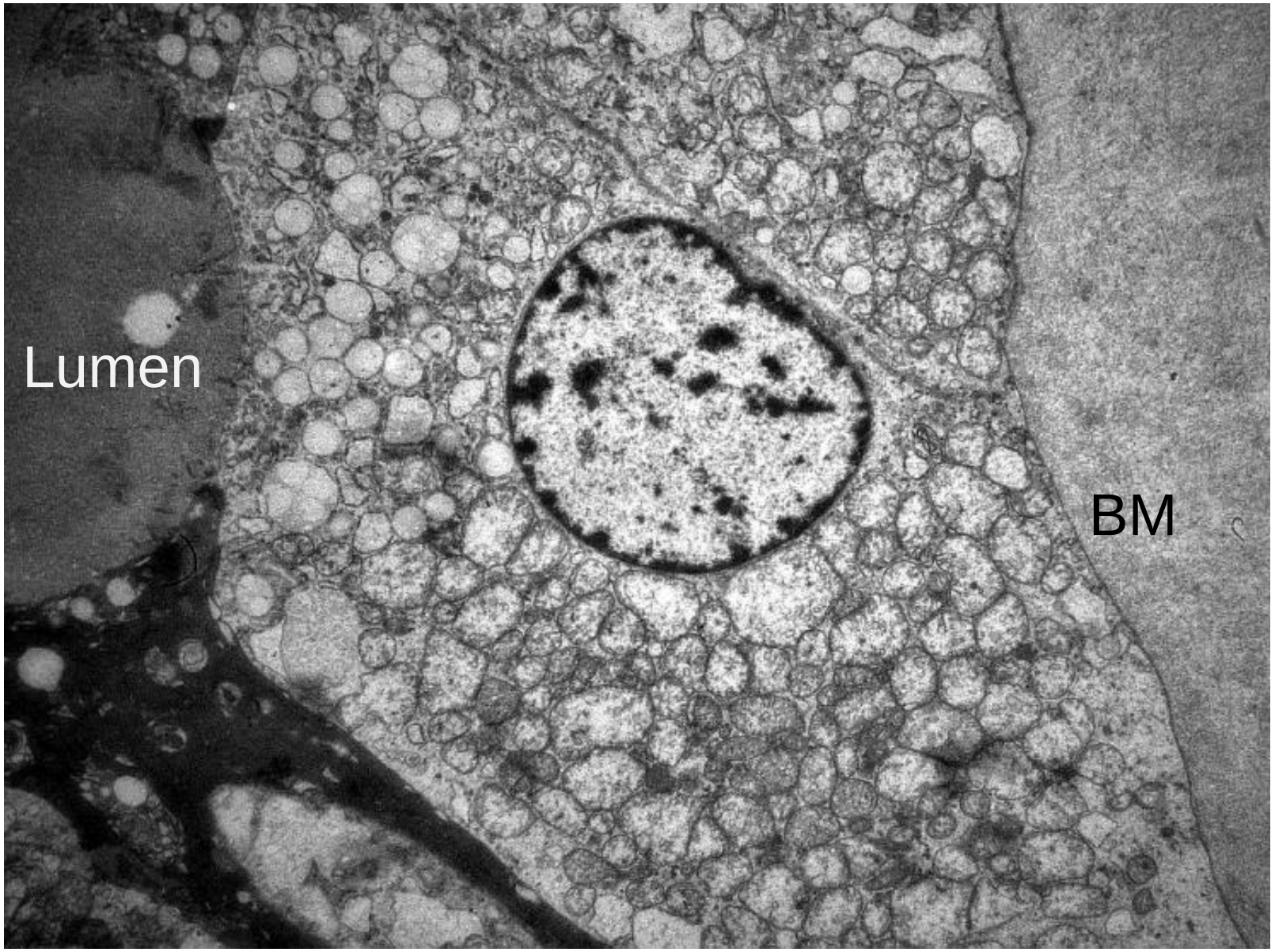
Mitochondria



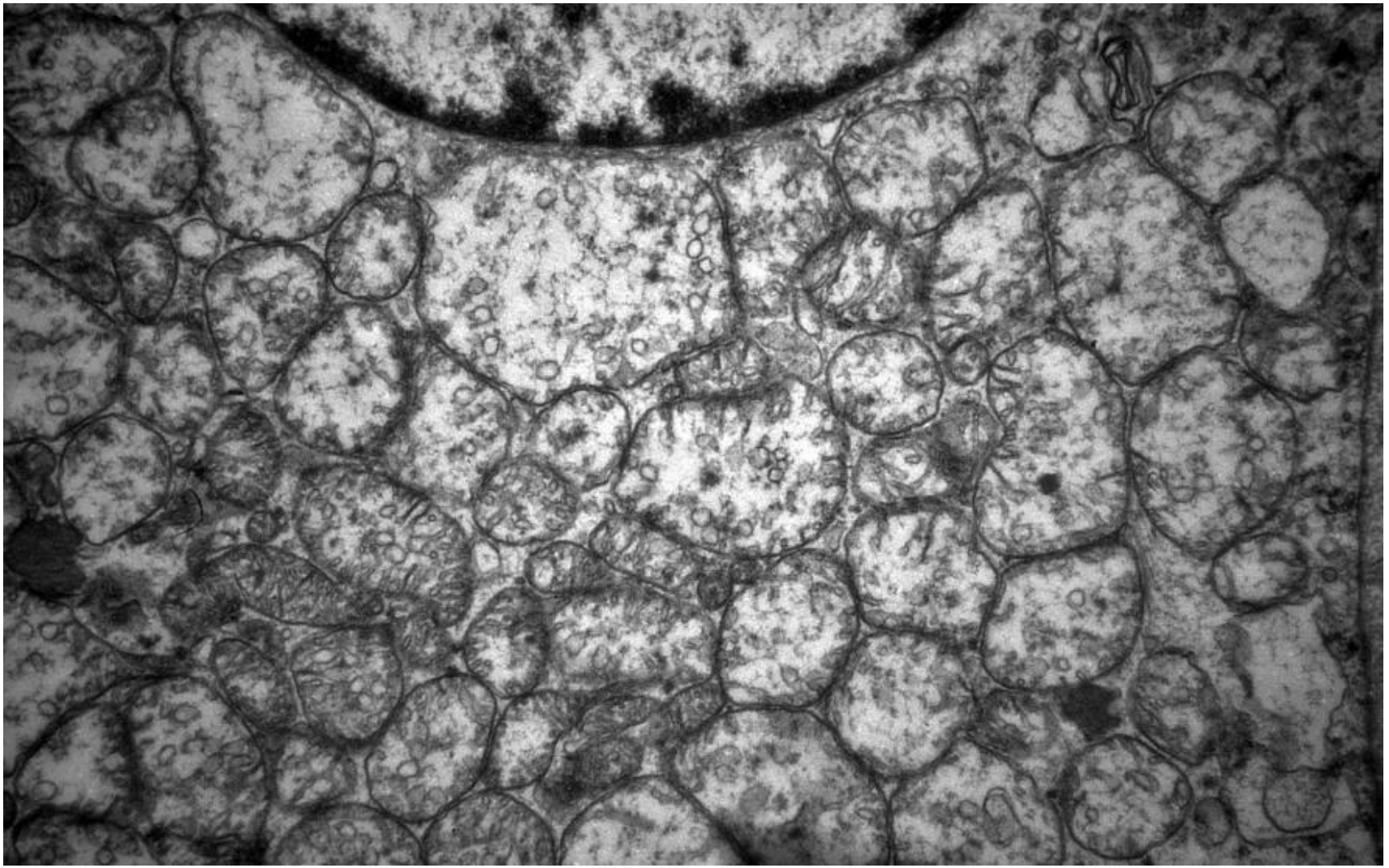
Polarized oncocytes



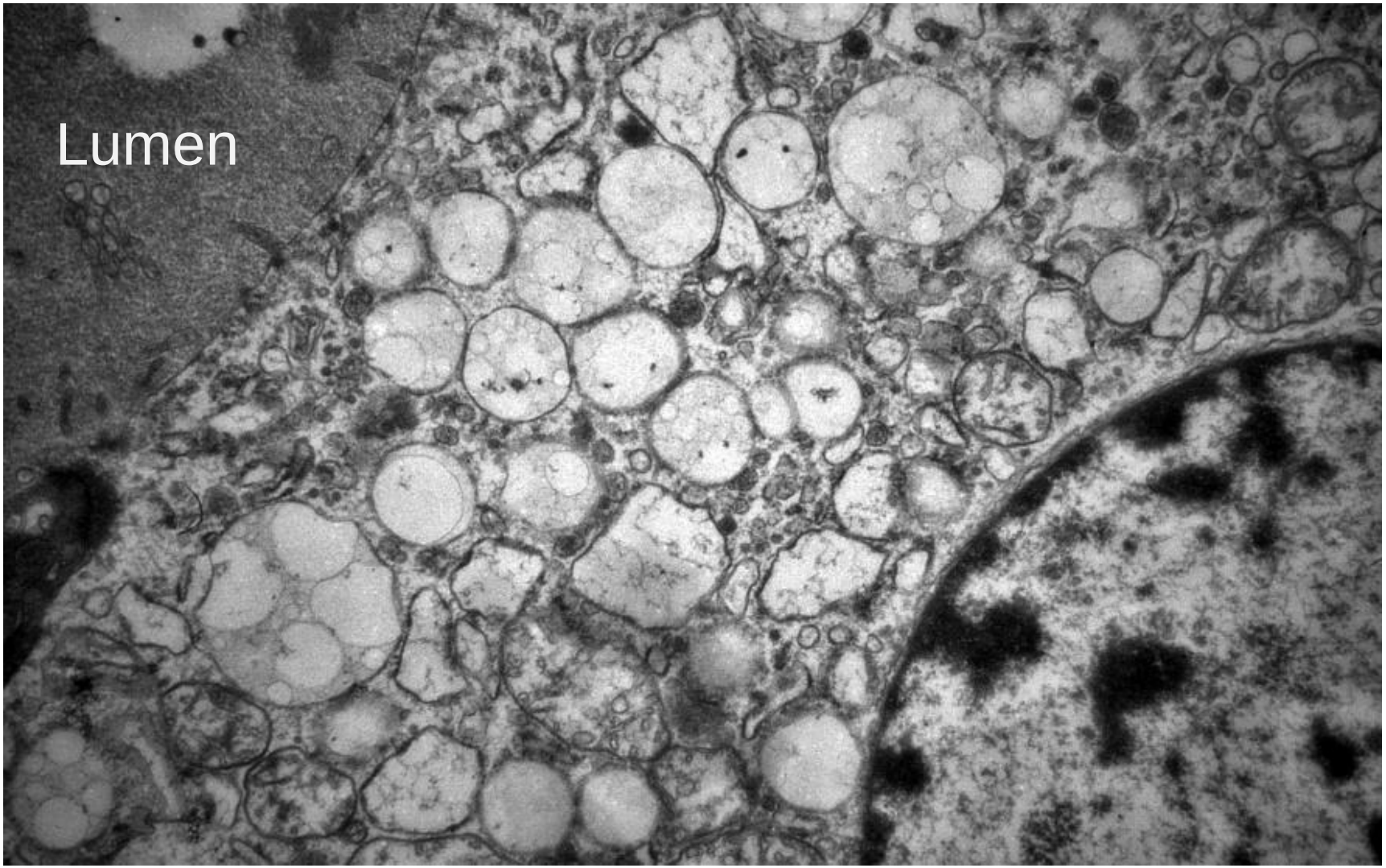
Polarized oncocyte



Polarized oncocyte: basal part



Polarized oncocyte: apical part

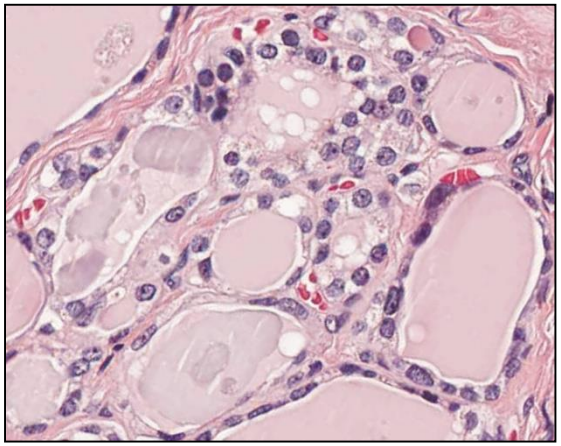
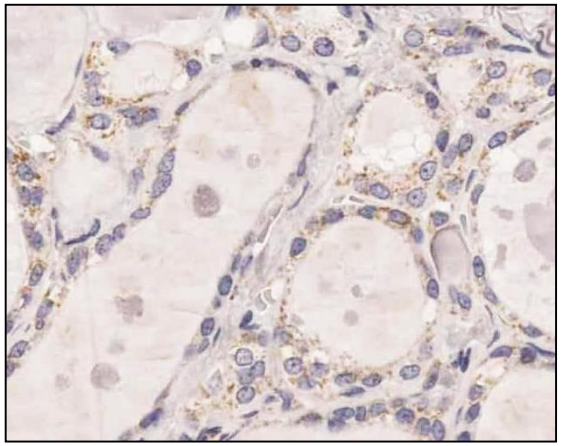
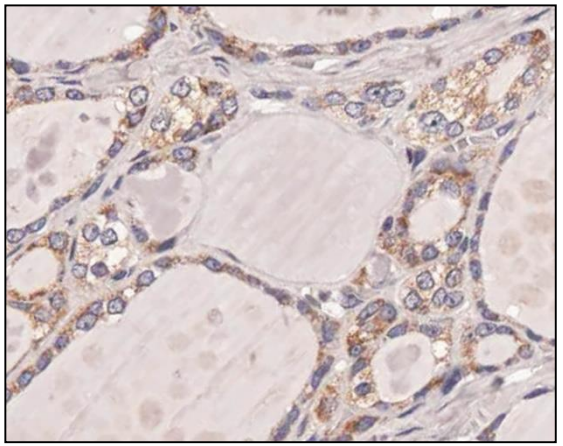
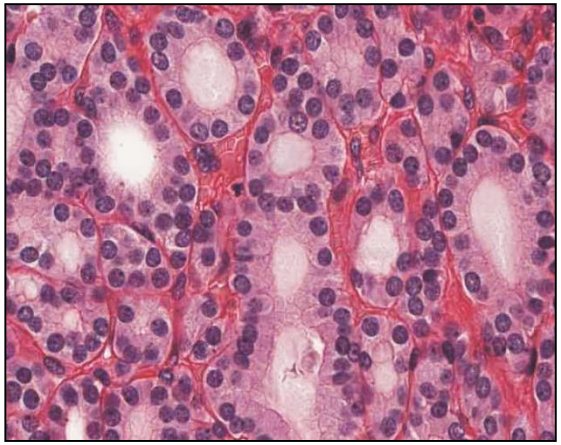
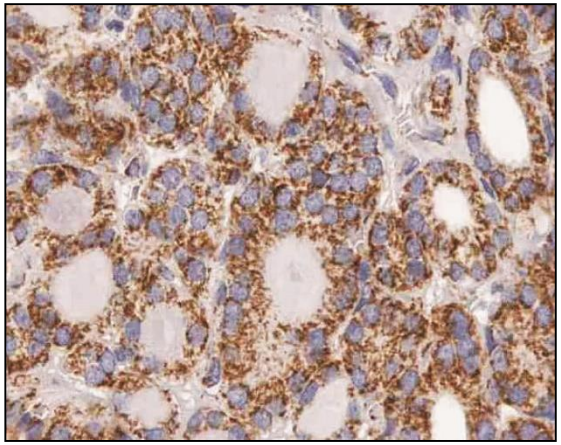
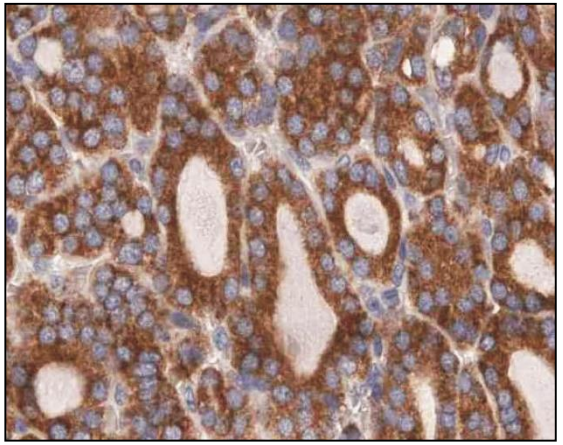


Endoplasmic reticulum marker

- Proteindisulfidisomerase (PDI), a resident RER protein
- Universally present in eukariotic cells
- Key enzyme catalysing formation and breakage of disulfide bonds
- Thus invovled in protein folding and chaperoning system

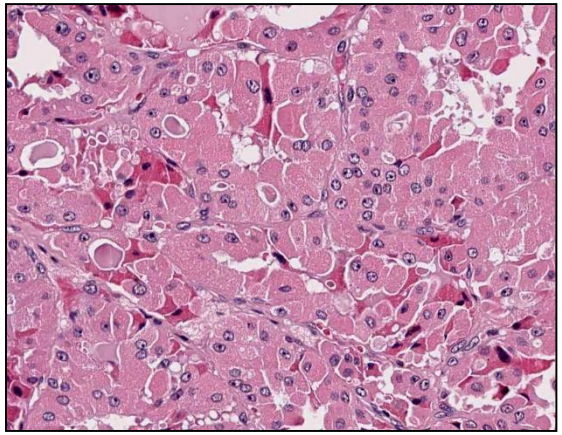
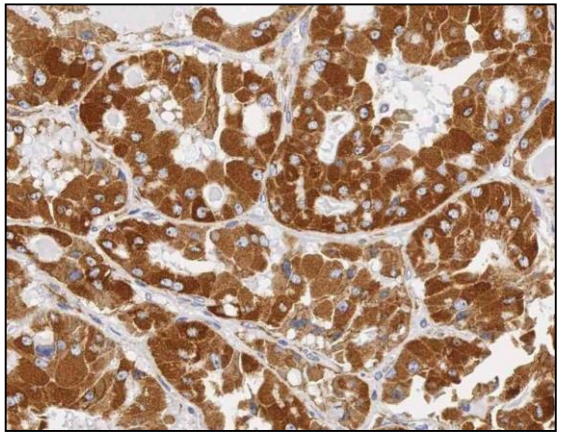
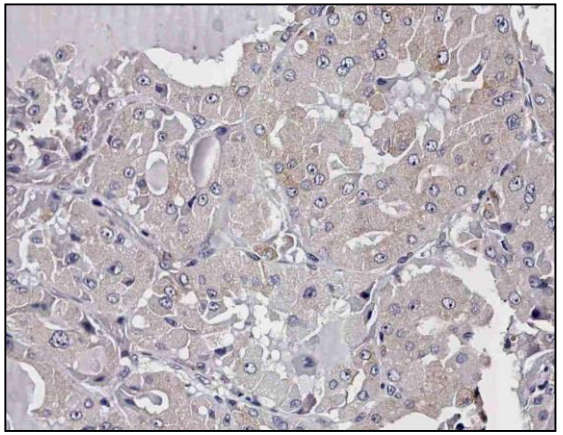
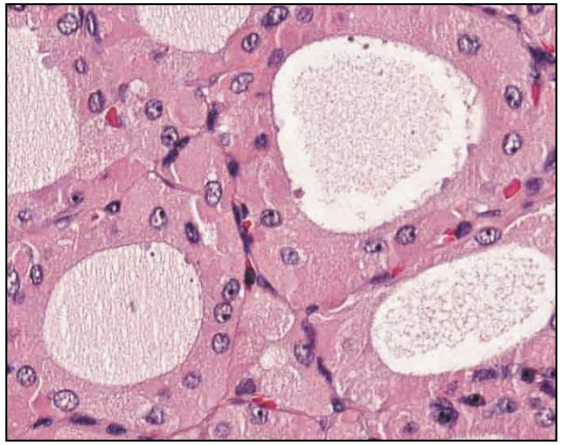
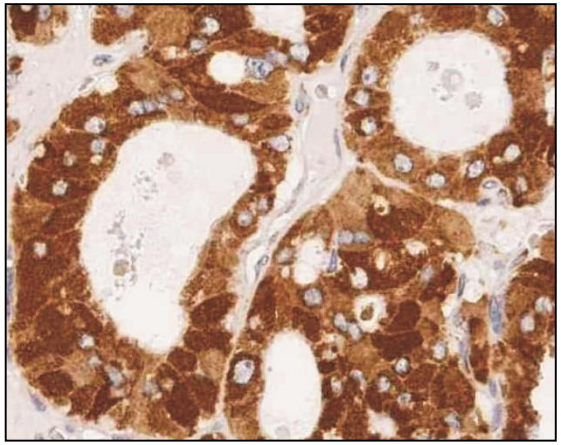
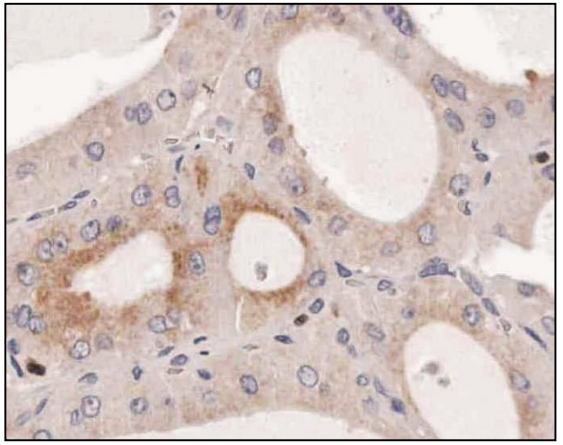
Non-oncocytic tissues 1

VINCENT ACADEMY OF PATHOLOGY

H&E	Mitochondria	Endoplasmic reticulum
		
		

Classic oncocytes

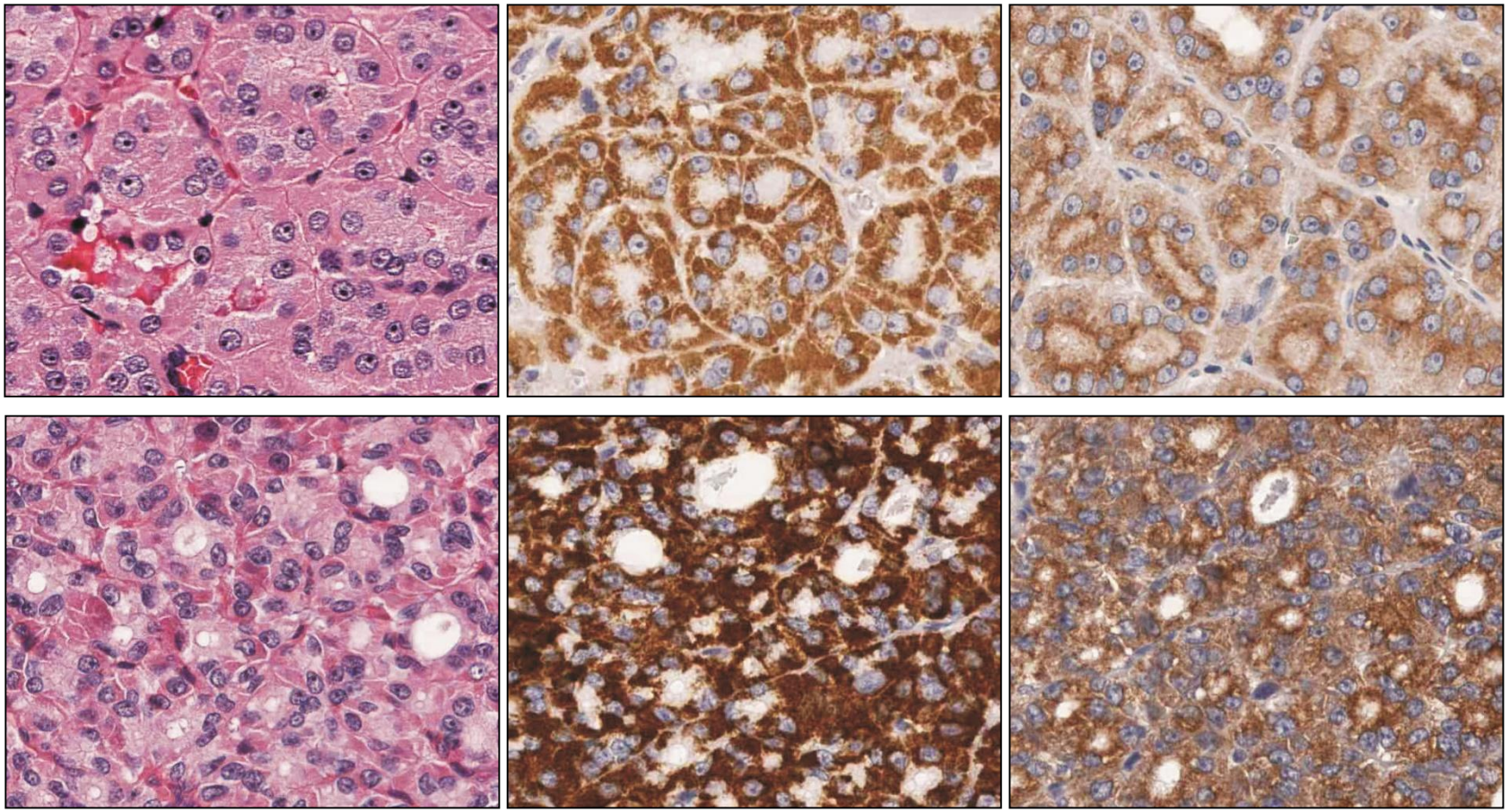
Medical University of Graz

H&E	Mitochondria	Endoplasmic reticulum
		
		

Polarized oncocytes

VINCENT ACADEMY OF PATHOLOGY

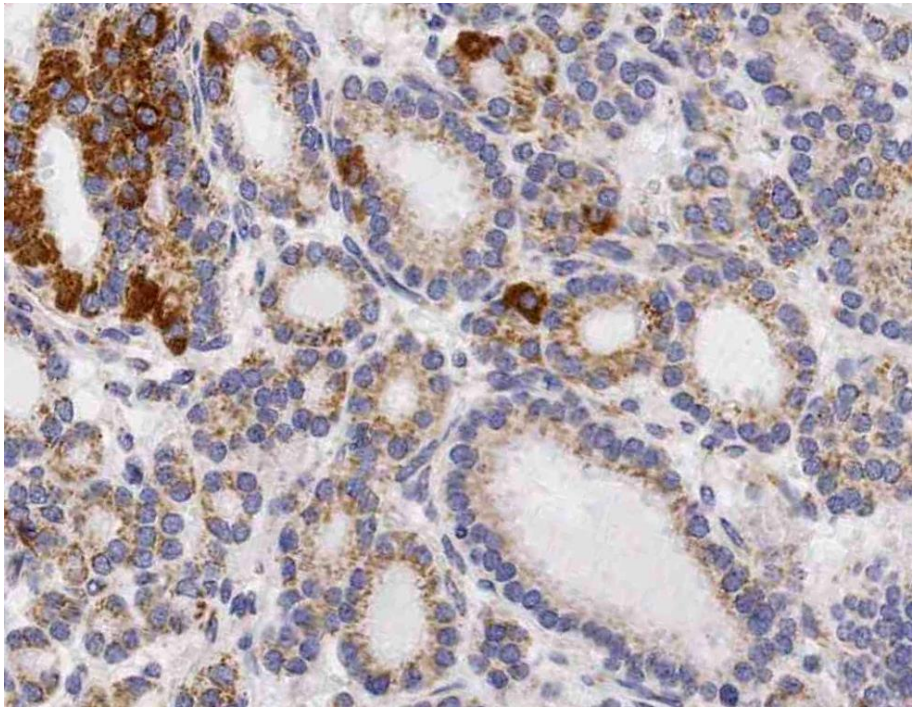
H&E | Mitochondria | Endoplasmic reticulum



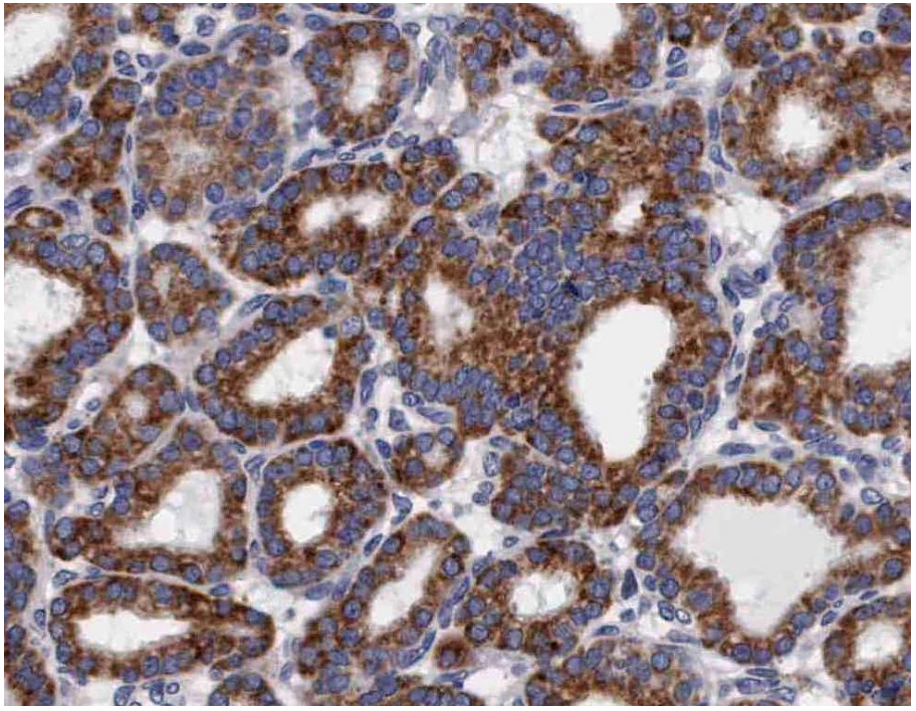
Toxic adenoma

VINCENT ACADEMY OF PATHOLOGY

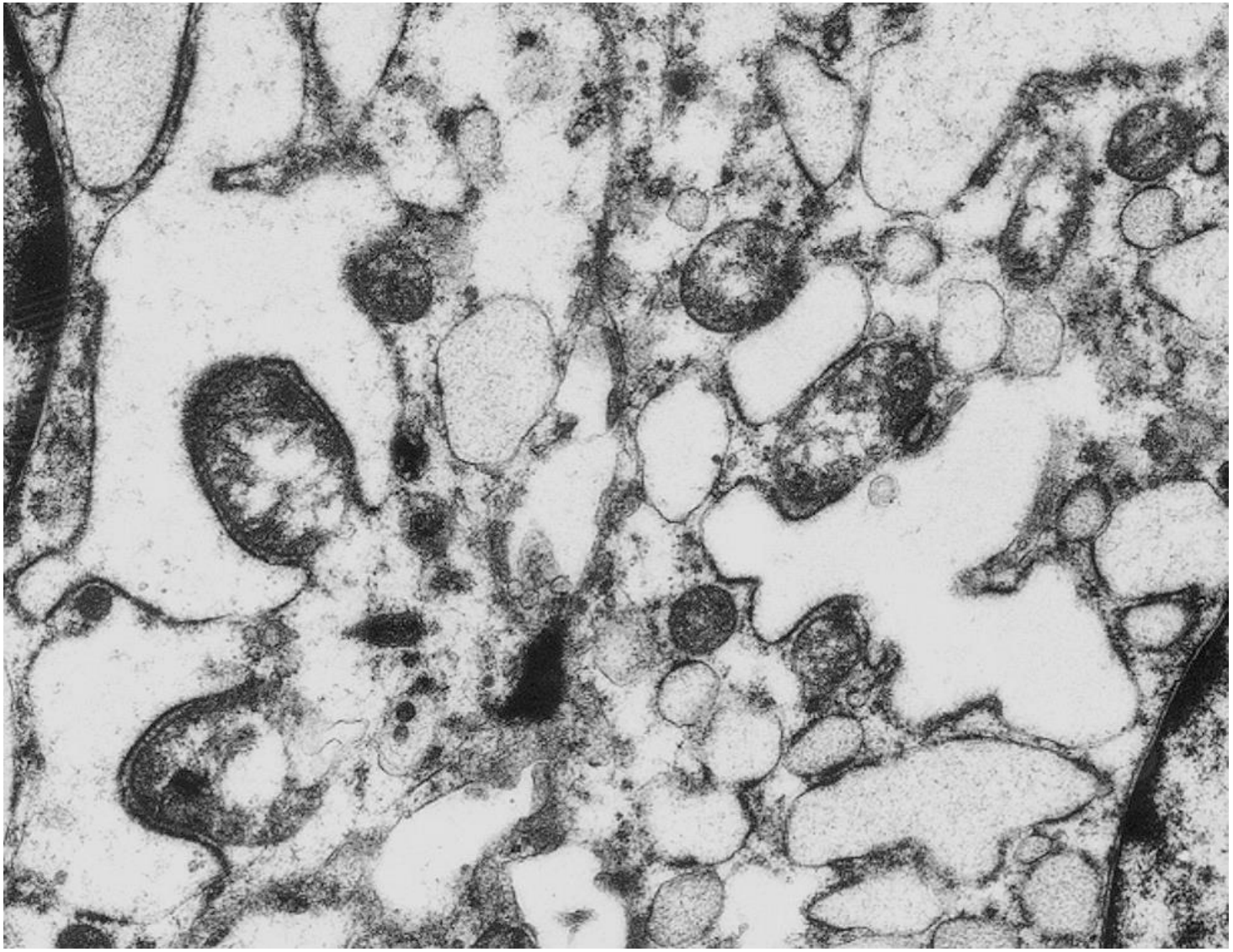
Mitochondria



Endoplasmic reticulum



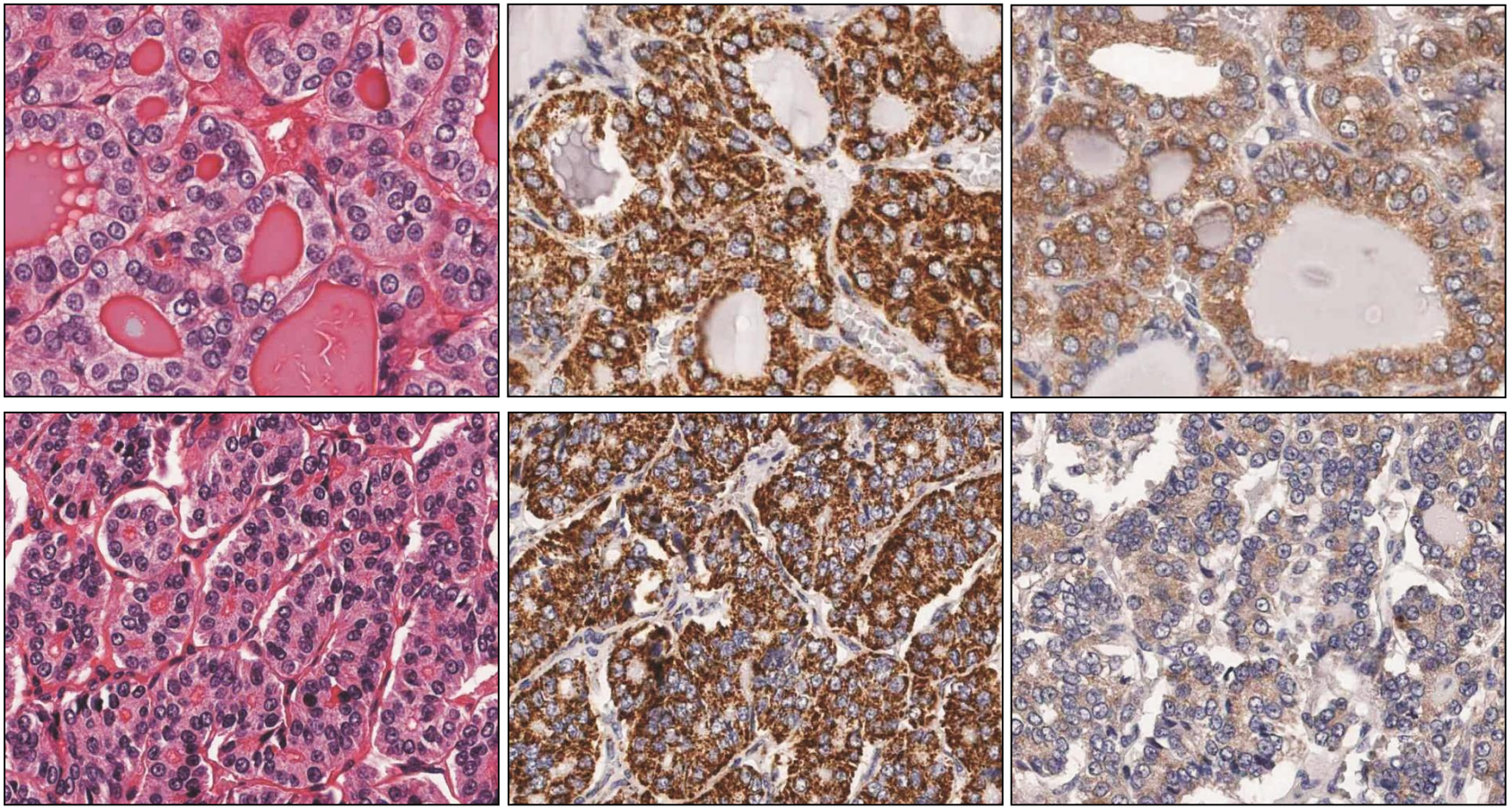
Toxic adenoma



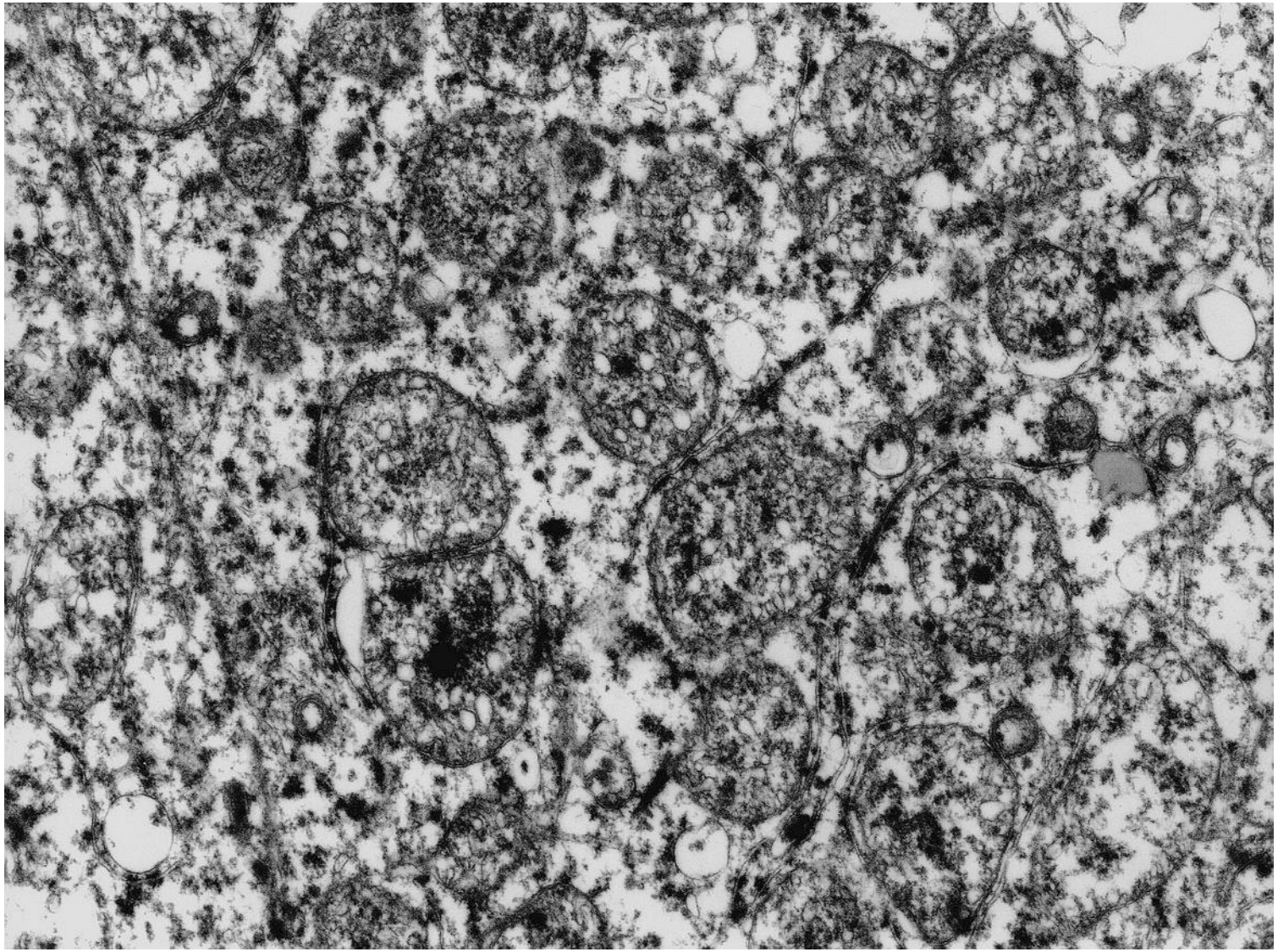
Non-oncocytic, but mitochondrion-rich

VINCENT ACADEMY OF PATHOLOGY

H&E | **Mitochondria** | **Endoplasmic reticulum**



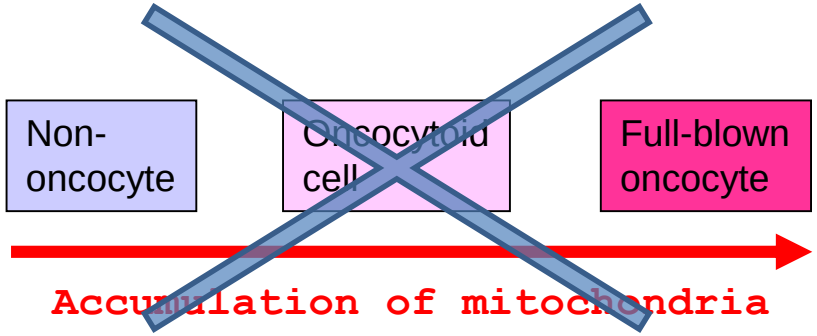
Non-oncocytic, but mitochondrion-rich

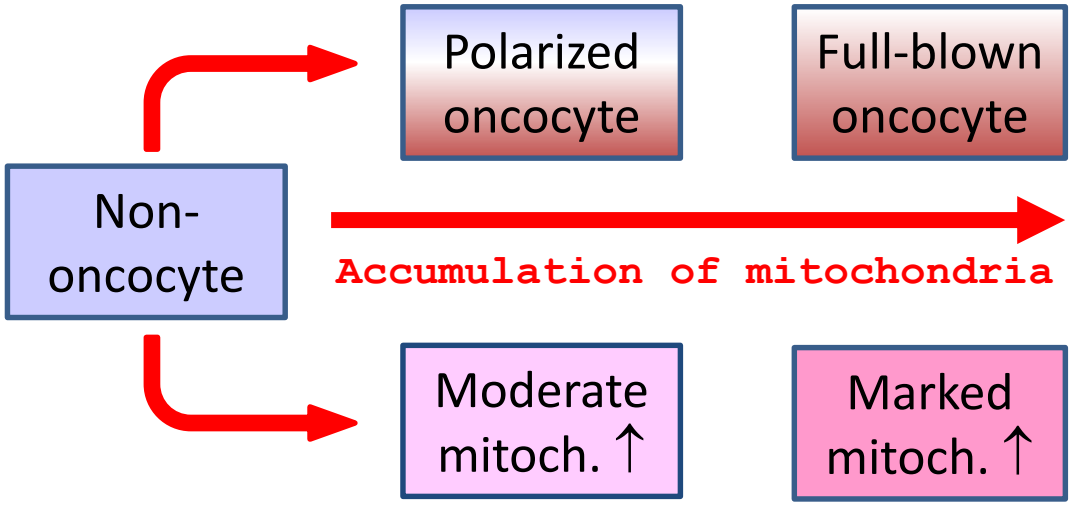


Main conclusions from our series

- The frequency of oncocytic change is badly underestimated, because it is often barely visible on HE (especially “polarized” oncocytes)
- The extent of cytoplasmic oxyphilia does not reflect the amount of mitochondria; it is actually impossible to estimate mitochondrial quantity based on HE
- The defining feature of oncocytic change is occurrence of certain areas within the cytoplasm (usually basal/lateral portions) with both accumulation of closely packed mitochondria and suppression of other organelles (which get displaced into other cytoplasmic areas, especially to the apex)
- There is also another type of mitochondrial accumulation which is obviously ***not*** related to oncocytic change

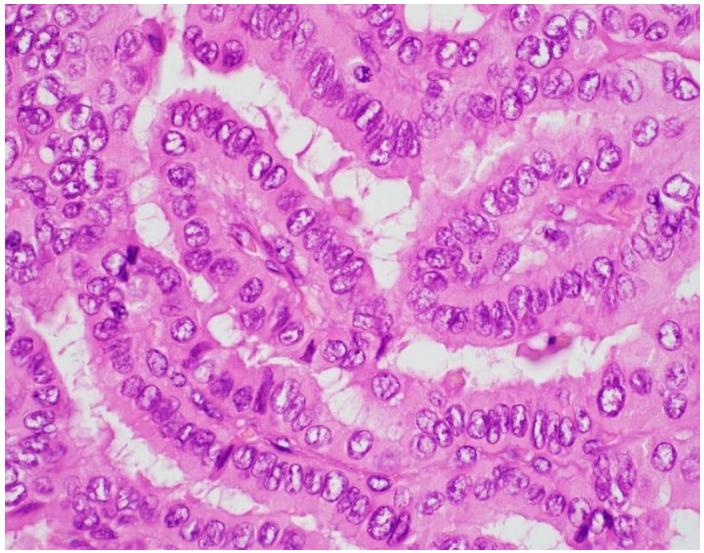
New concept of mitochondrial accumulation

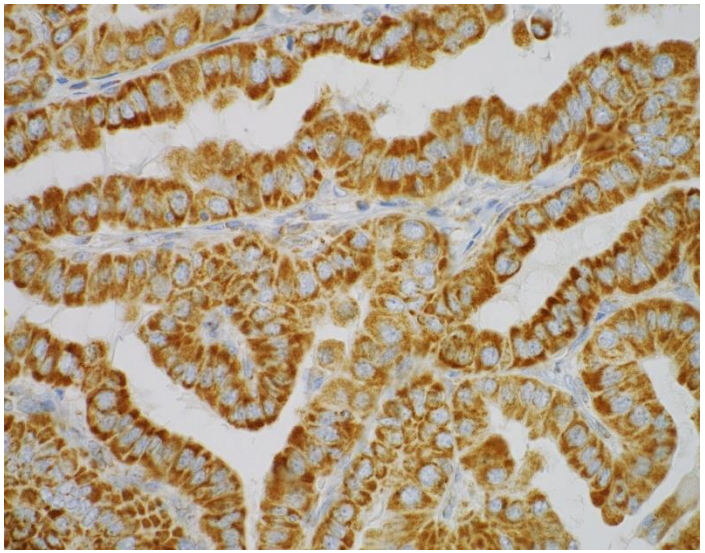




Still problems

- Staining patterns sometimes difficult to asses
- Polarity does not help in solid lesions
- Prohobitin/PDI duo failed to work in other organs (parathyroid, renal oncocytomas)
- Peculiar “bipolar” mitochondrial staining pattern in PTC

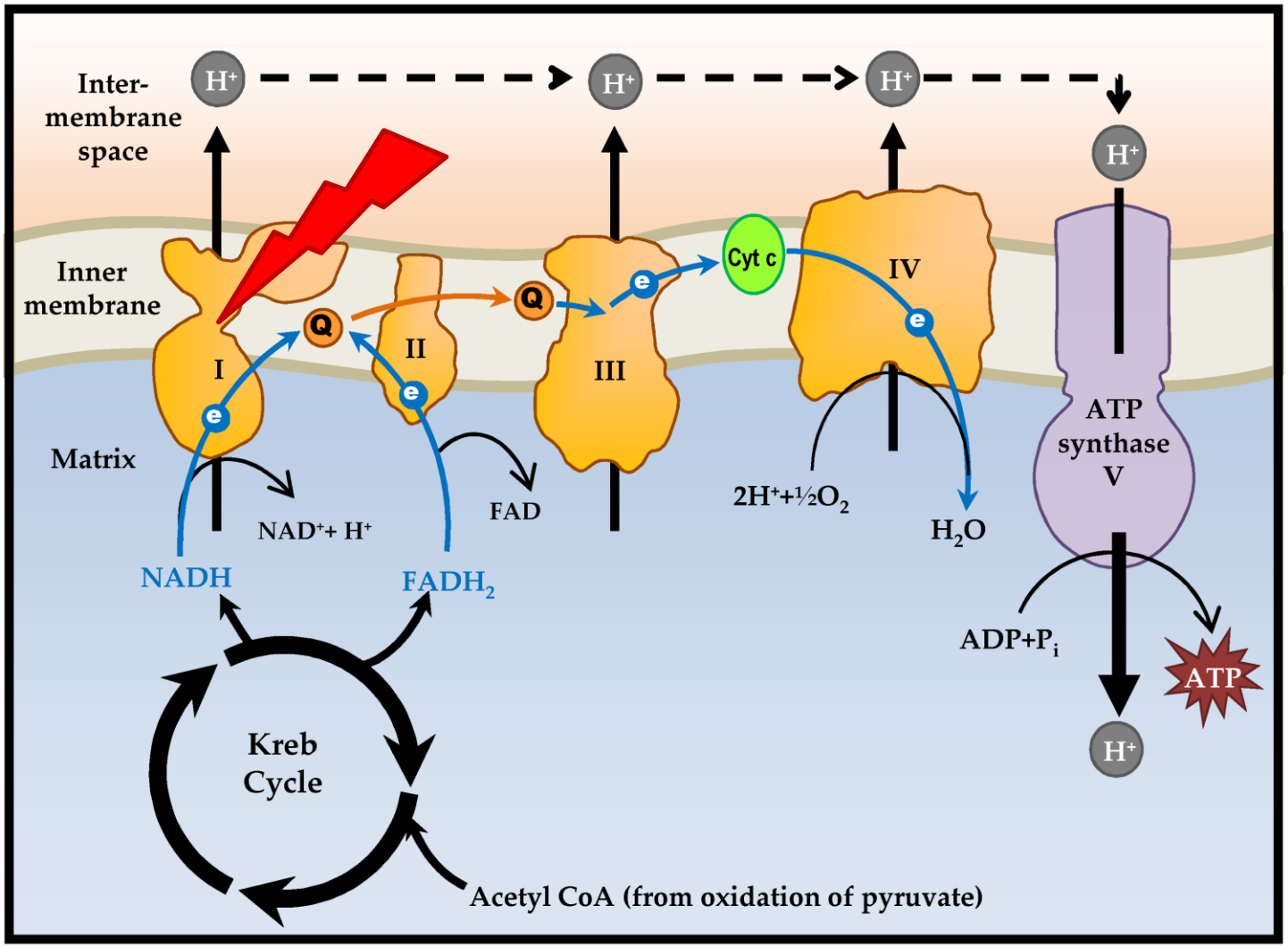




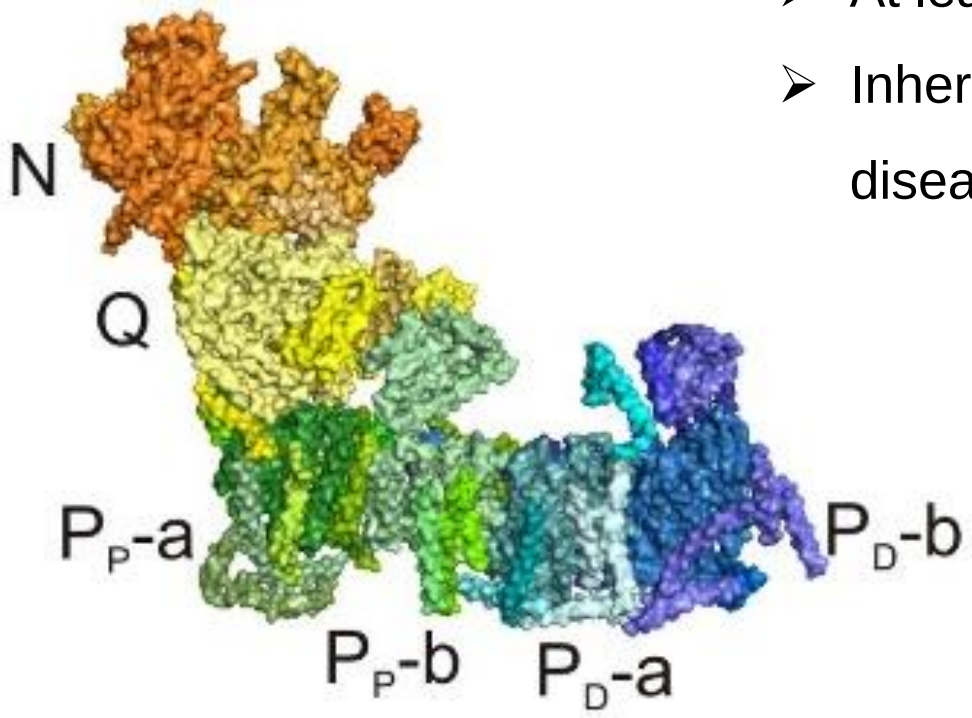
Pathogenesis-based solution

- Coming back to the original hypothesis by Tremblay and Pearse: "*increased formation of biochemically defective mitochondria due to an increased demand*"
- Savanger et al, 2001: ATP synthesis was significantly lower in all 22 studied oncocytic tumors, compared with controls, suggesting that a **coupling defect in OXPHOS** may be a cause of mitochondrial hyperplasia in oxyphilic thyroid tumors
- In 2000s: increasing evidence of genetic alterations in mitochondrial DNA and especially genes encoding **complex I** (and also III and IV) of ETC (Porto, Bologna, Salzburg groups)

Mitochondrial energy production by OXPHOS



Complex I

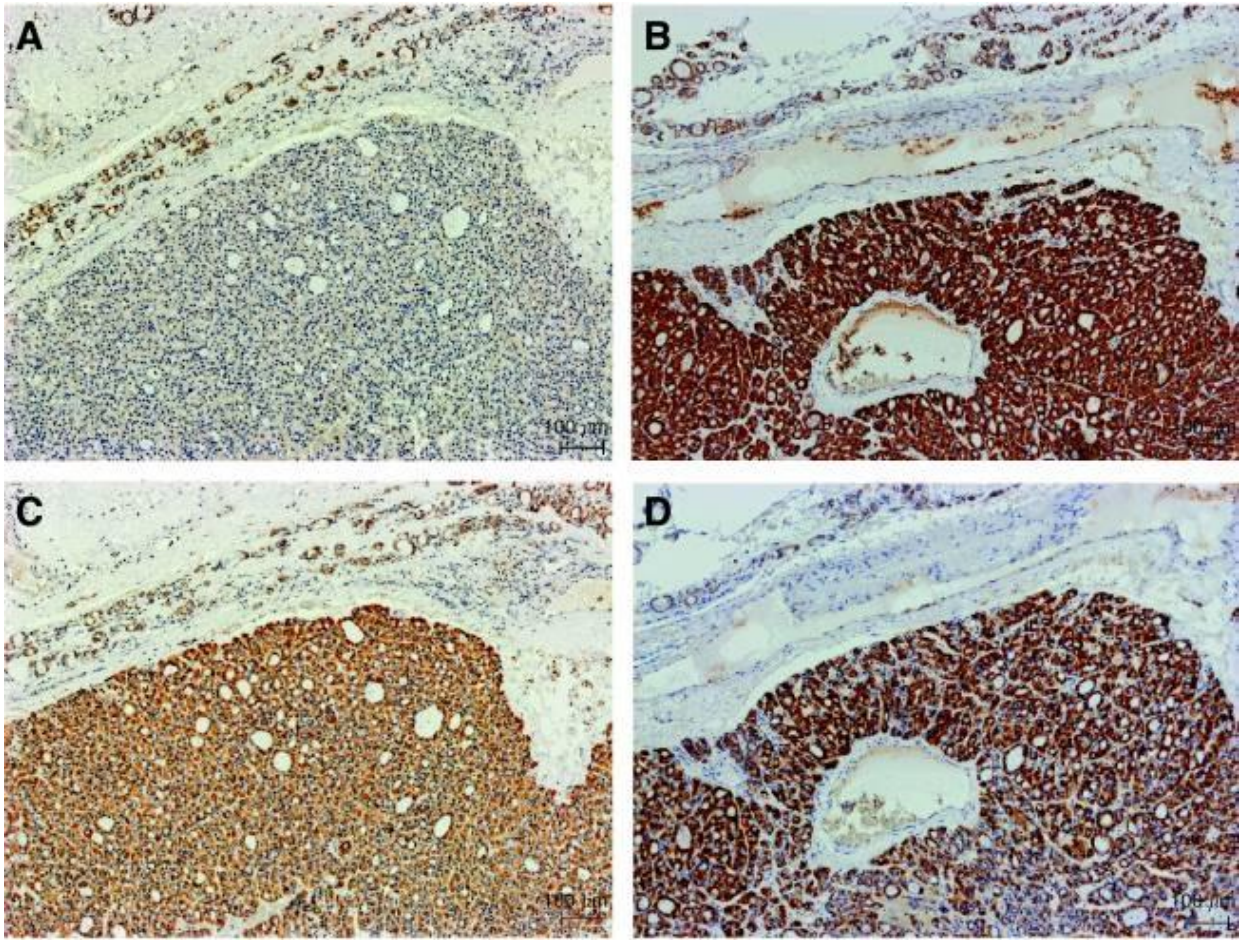


- One of the largest known macromolecular complexes
- 44 structural subunits
- At least 13 assembly factors
- Inherited defects -> mitochondrial diseases (e.g. Leigh syndrome)

NDUFS-4 as a surrogate complex I marker

"NDUFS-4 subunit is particularly unstable and its stability depends on the presence of preassembled complex I"

C-I



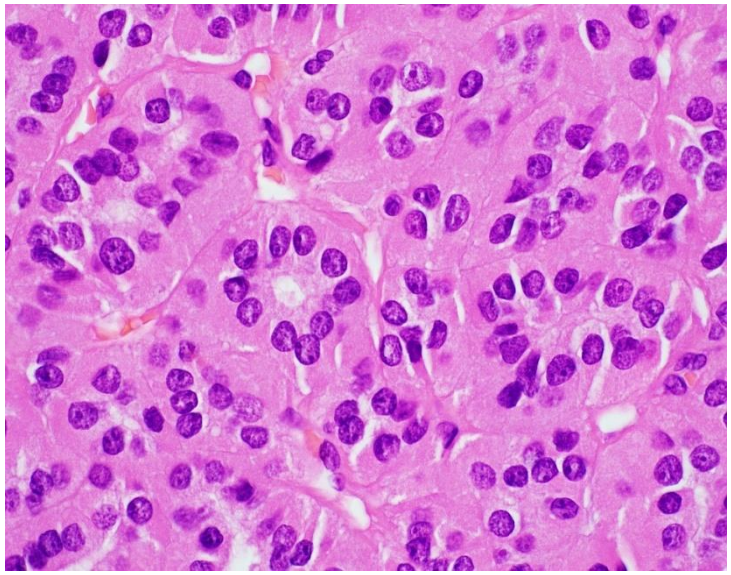
C-II

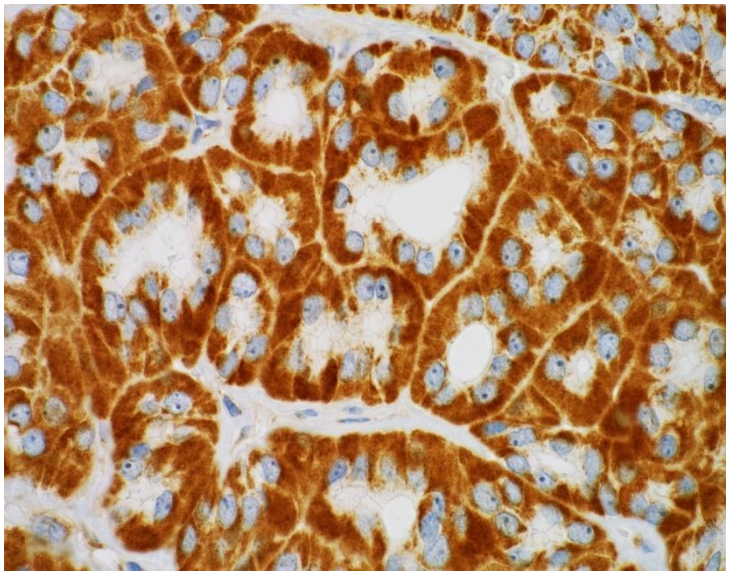
C-III

C-IV

From: Zimmermann et al, Br J Cancer (2009) 100, 1434-1437

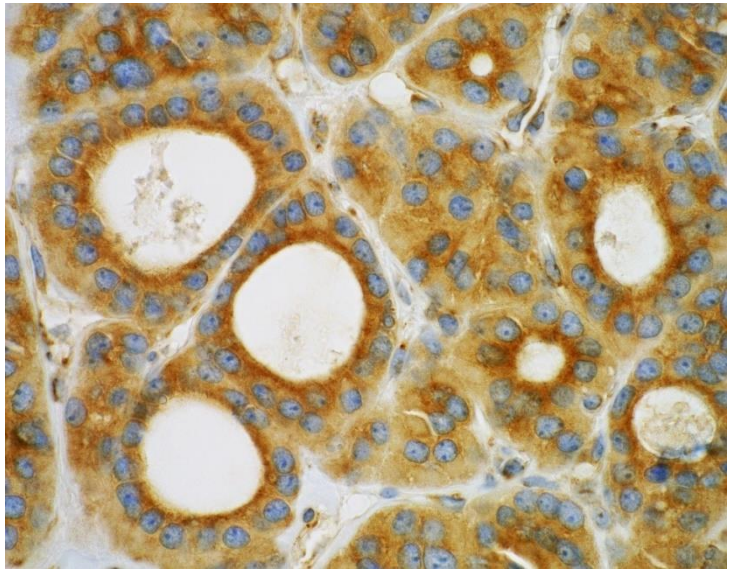
Polarized oncocytes

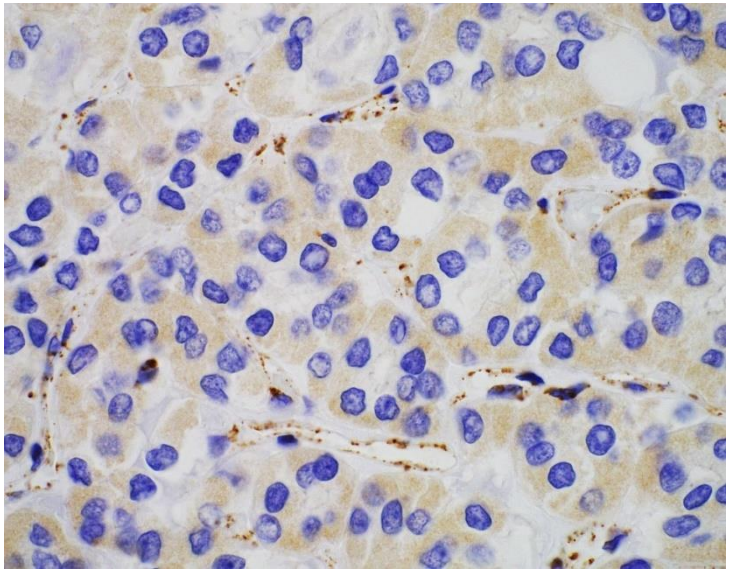




Prohibitin

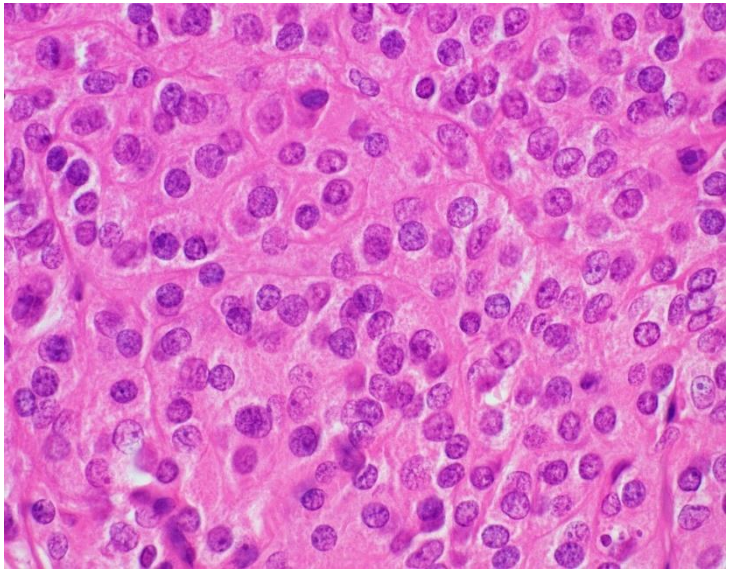
PDI



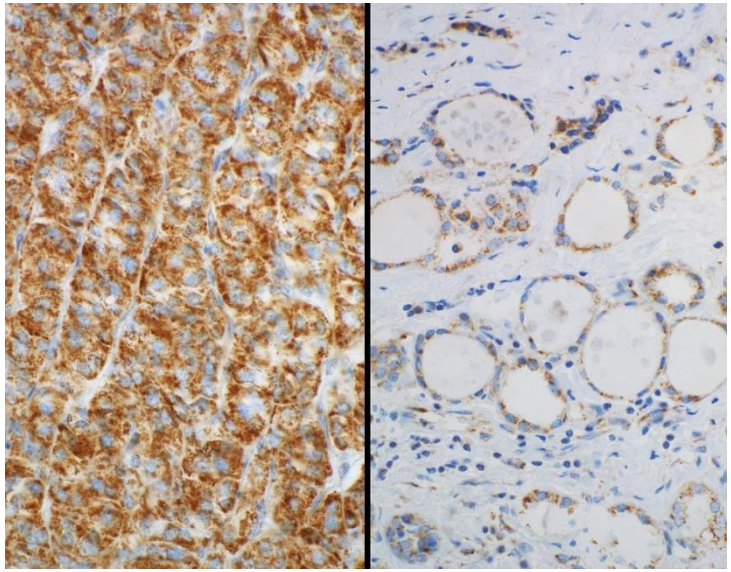


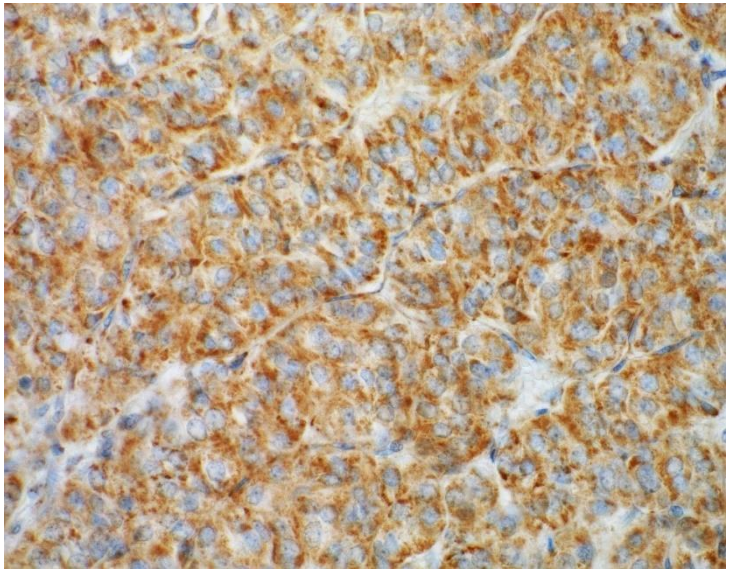
NDUFS-4

Mitochondrion-rich non-oncocytes



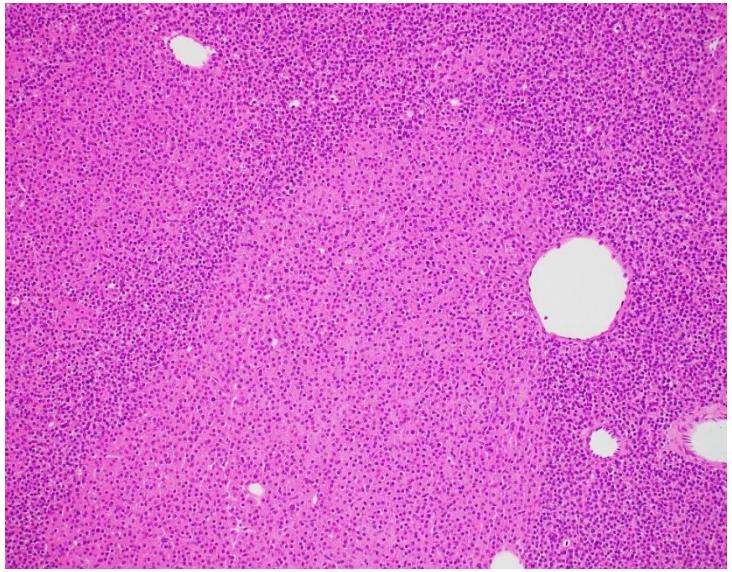
Prohibitin



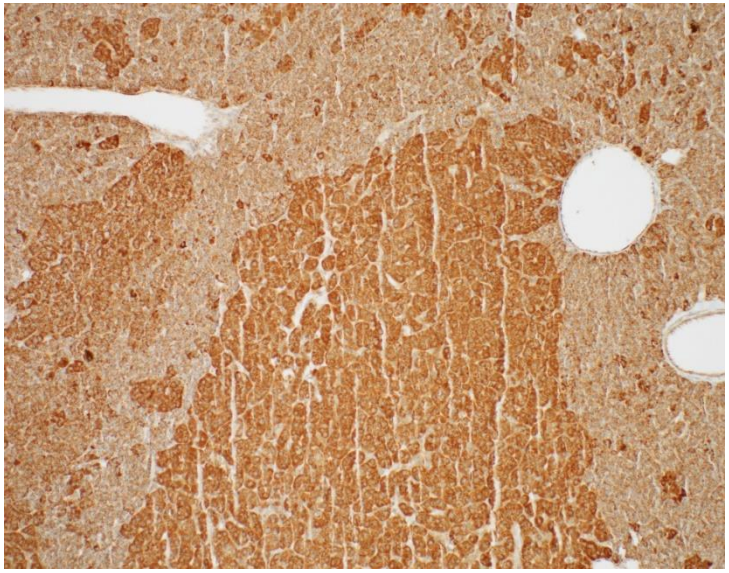


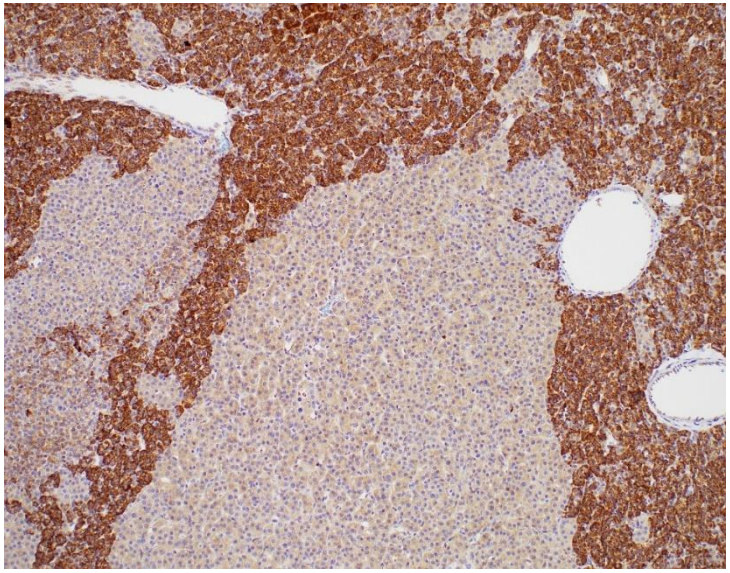
NDUFS-4

Parathyroid adenoma



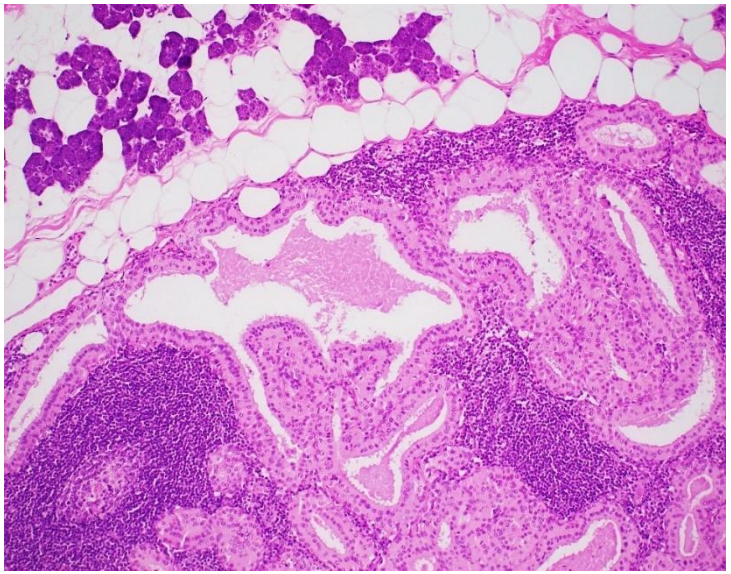
Prohibitin





NDUFS-4

Warthin tumor



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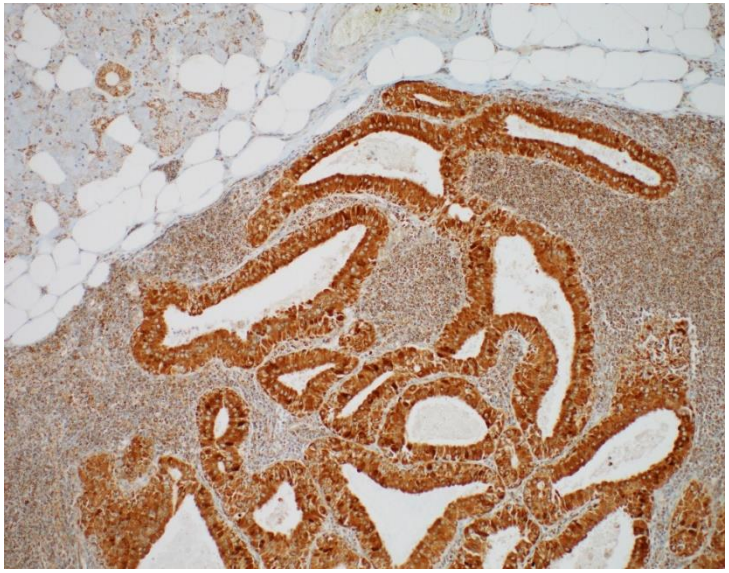
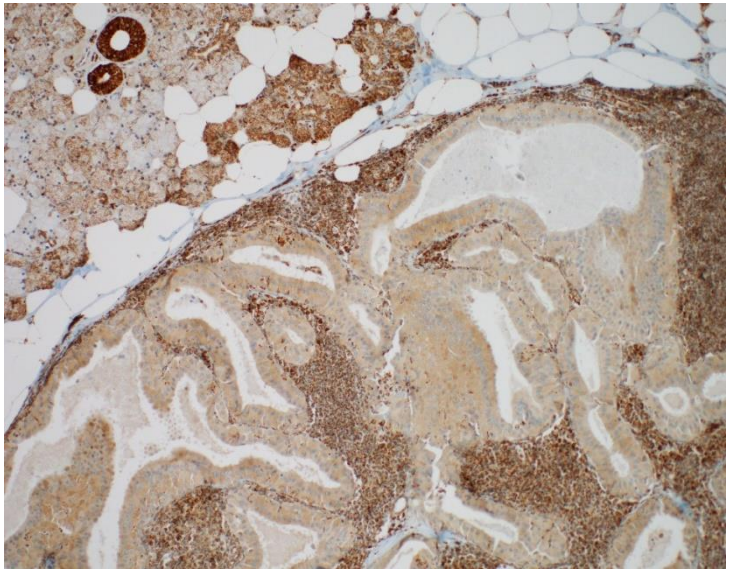
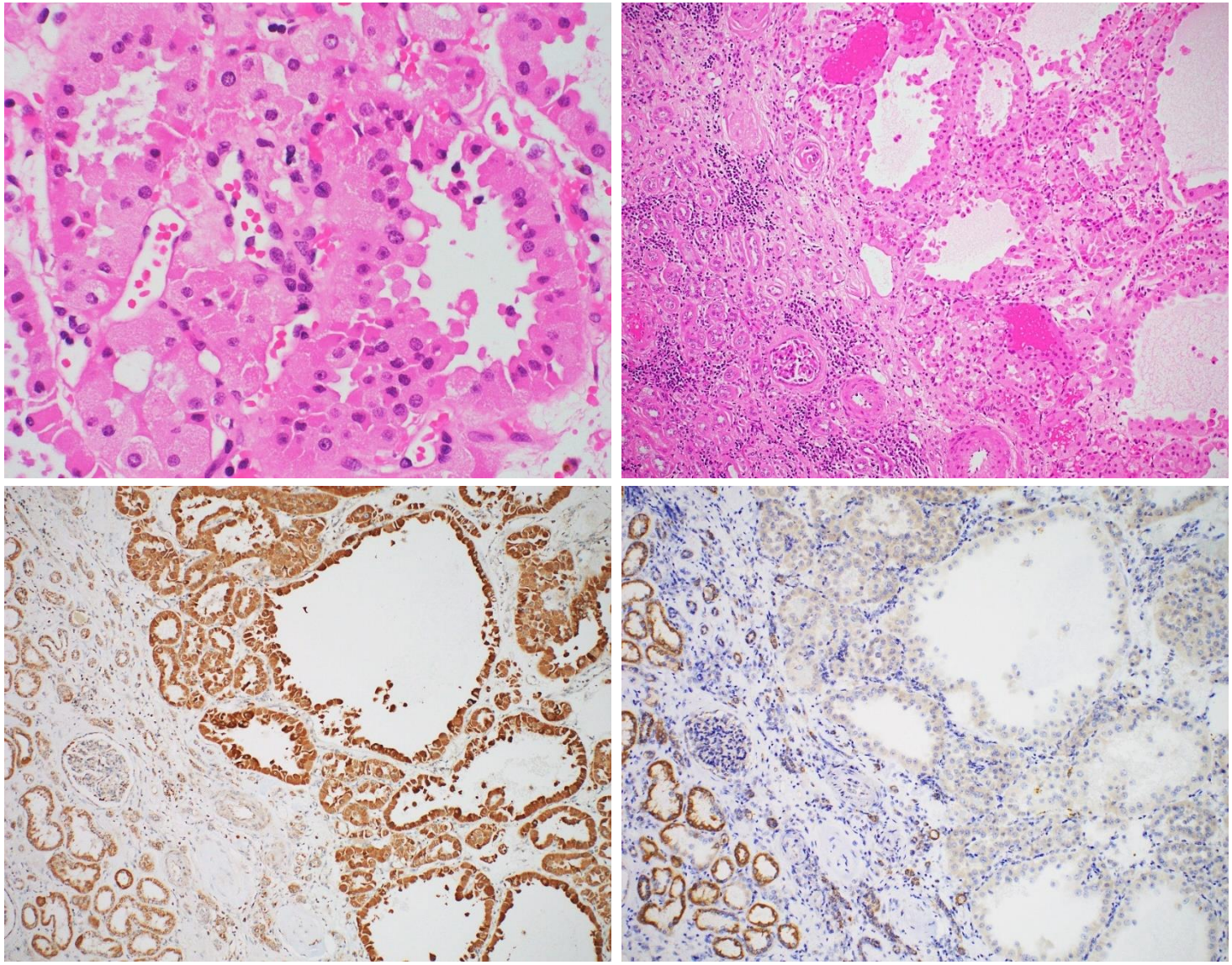




NDUFS-4

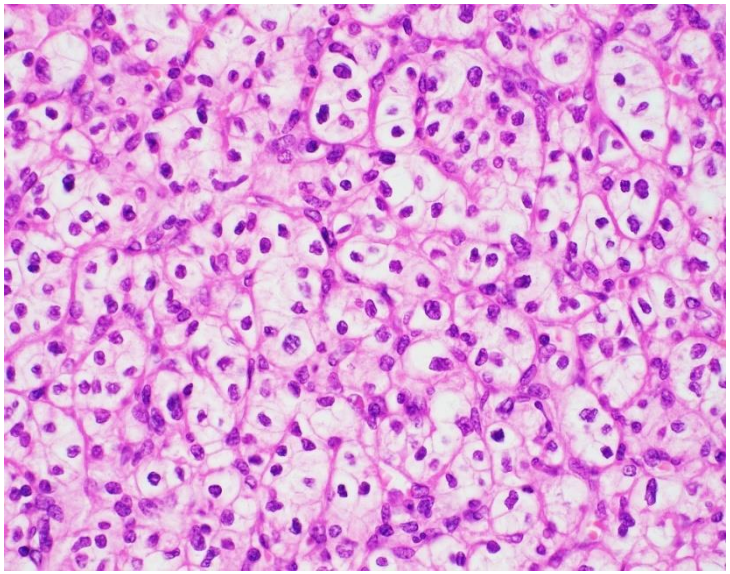
Renal oncocytoma



Prohibitin

NDUFS-4

Caveats: clear cell RCC



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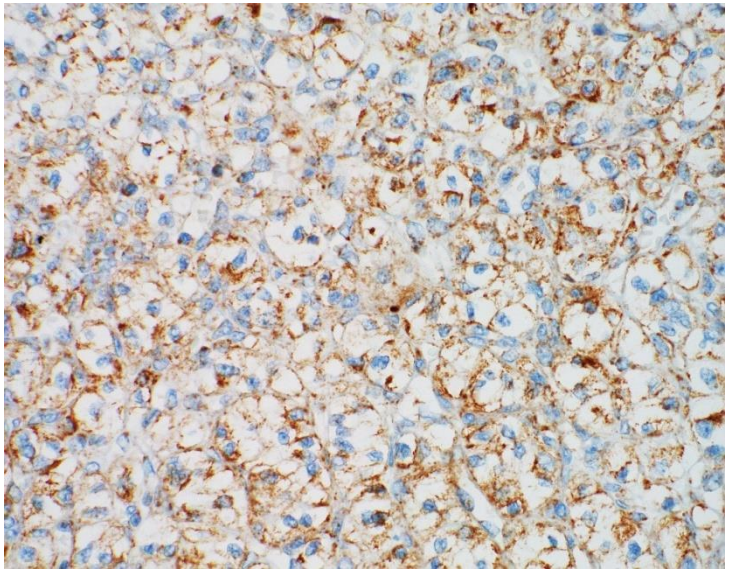
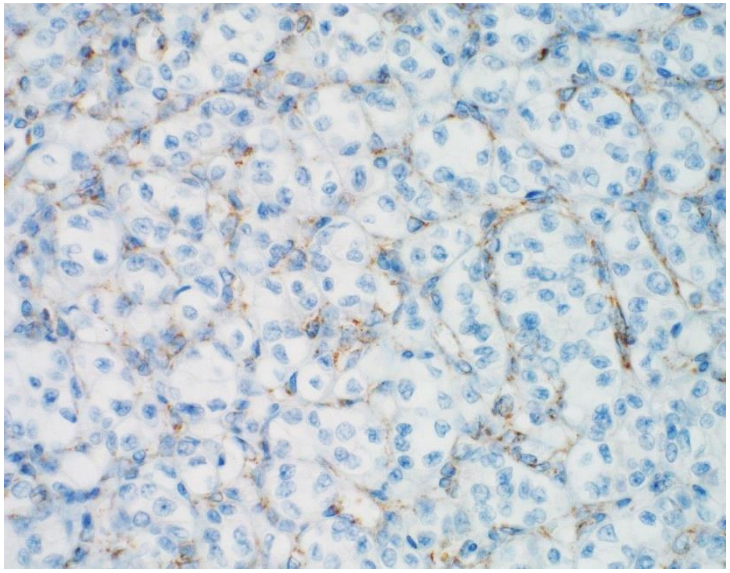
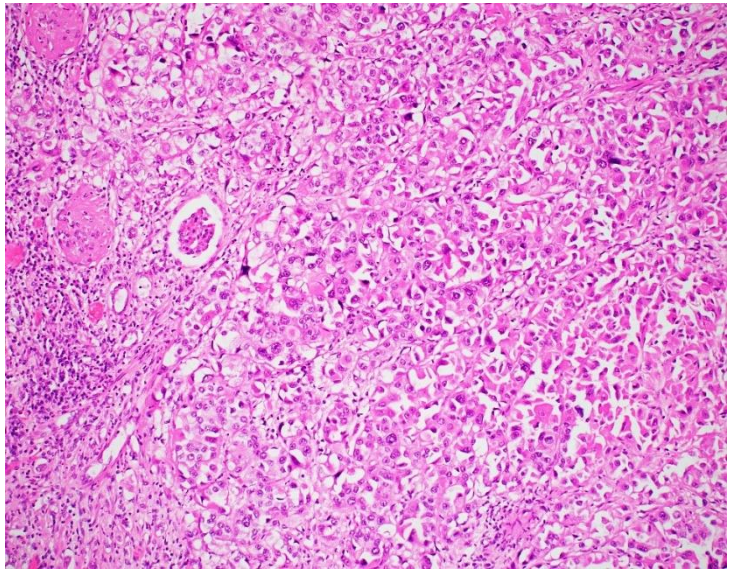
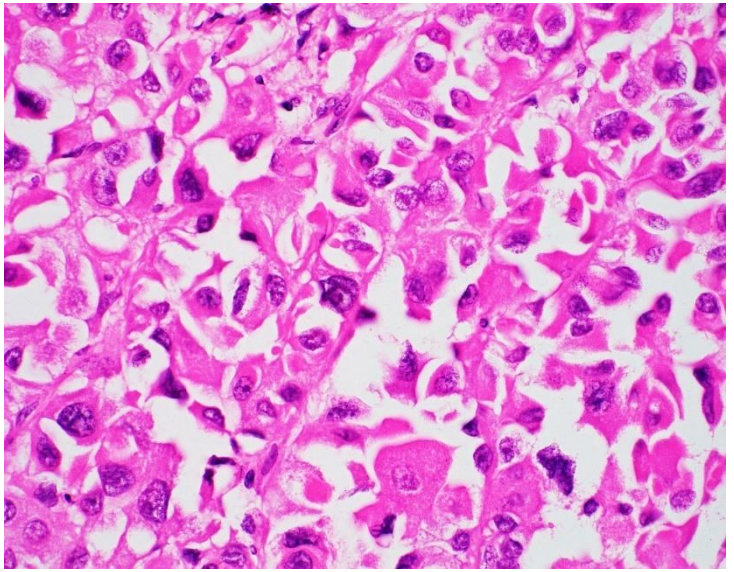




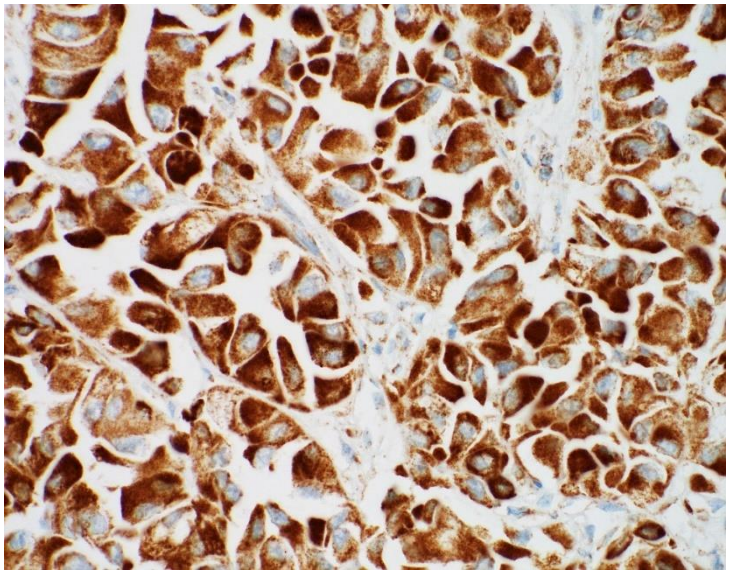
NDUFS-4

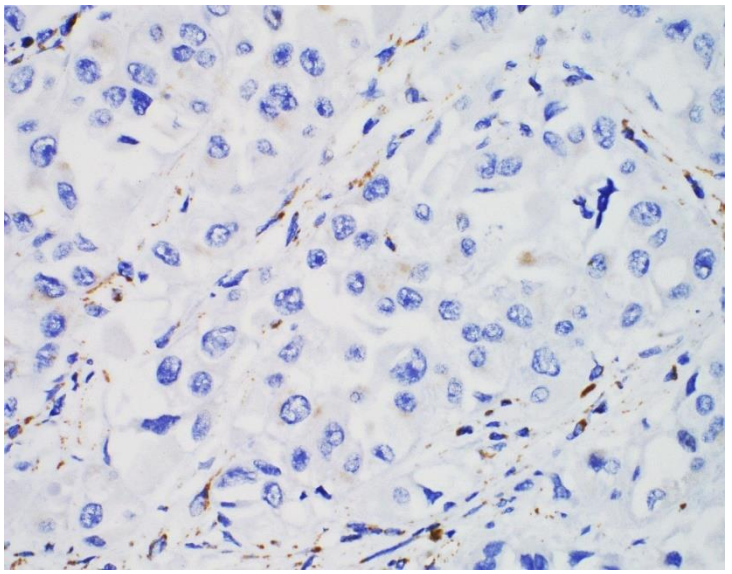
Even more caveats: oncocytiod urothelial Ca





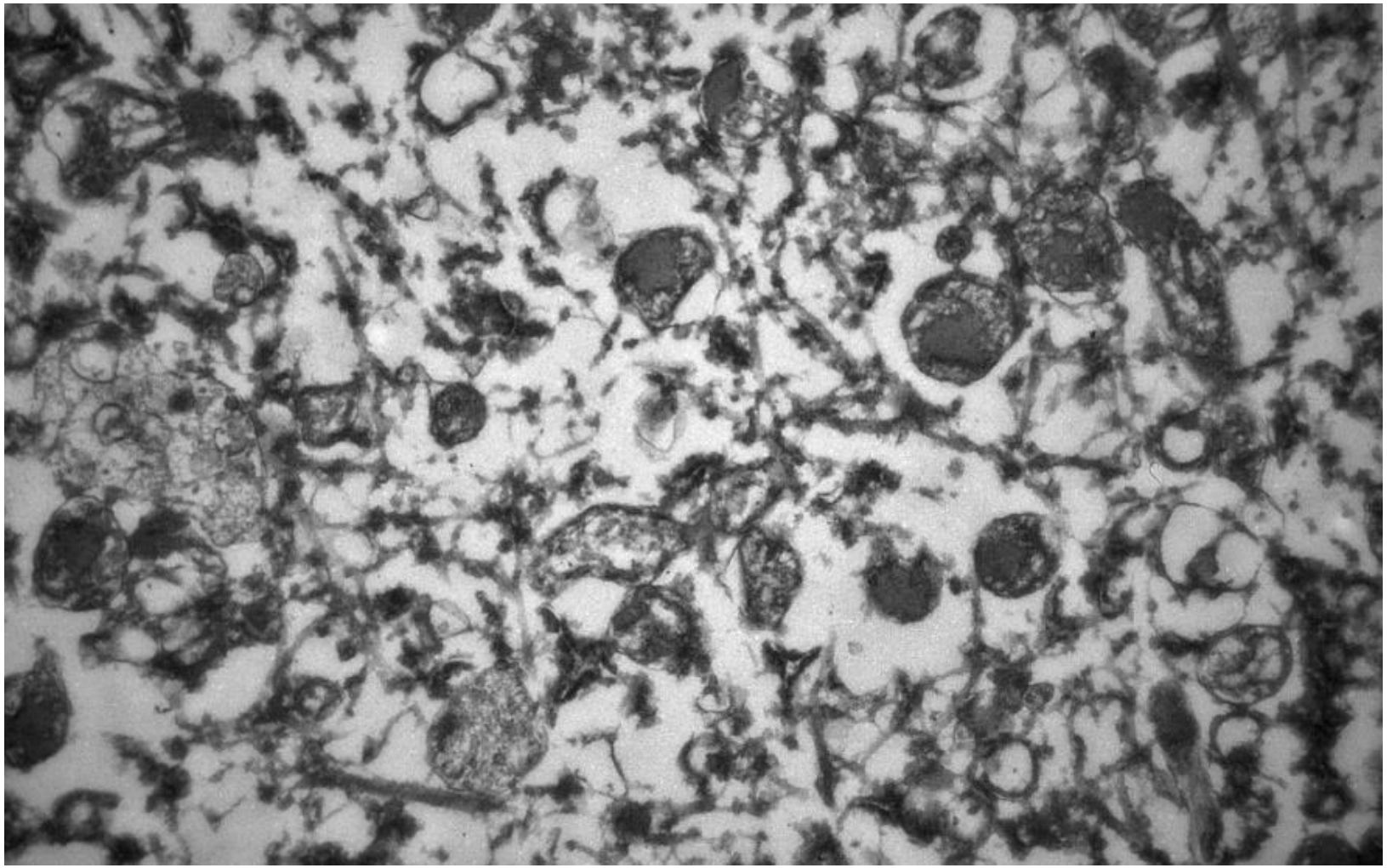
Prohibitin





NDUFS-4

Even more caveats: oncocytiod urothelial Ca

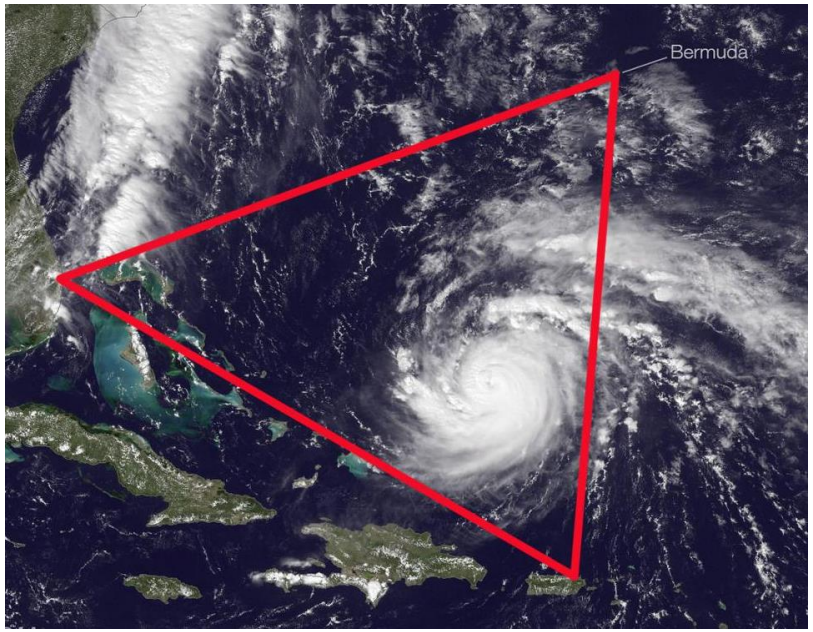


Is this only complex I ?

- By far not all cells with defective complex I are oncocytes
- All oncocytes have defective complex I, but the extent of oncocytic change within the cytoplasm can vary greatly
- Thus, there must be something else:
  - Either different functional grades of complex I impairment
  - Or defects in other components (complex III and IV defects also reported, the interplay between the complexes of ETC probably also important ("respirosome" concept)

Bermuda triangle in the cytoplasmic „sea“

Mitochondrion-rich:
- Prohibitin high (roughly granular)
- NDUFS-4 pos.



Mitochondrion-poor:
- Prohibitin low
- NDUFS-4 pos.

What is it all good for?

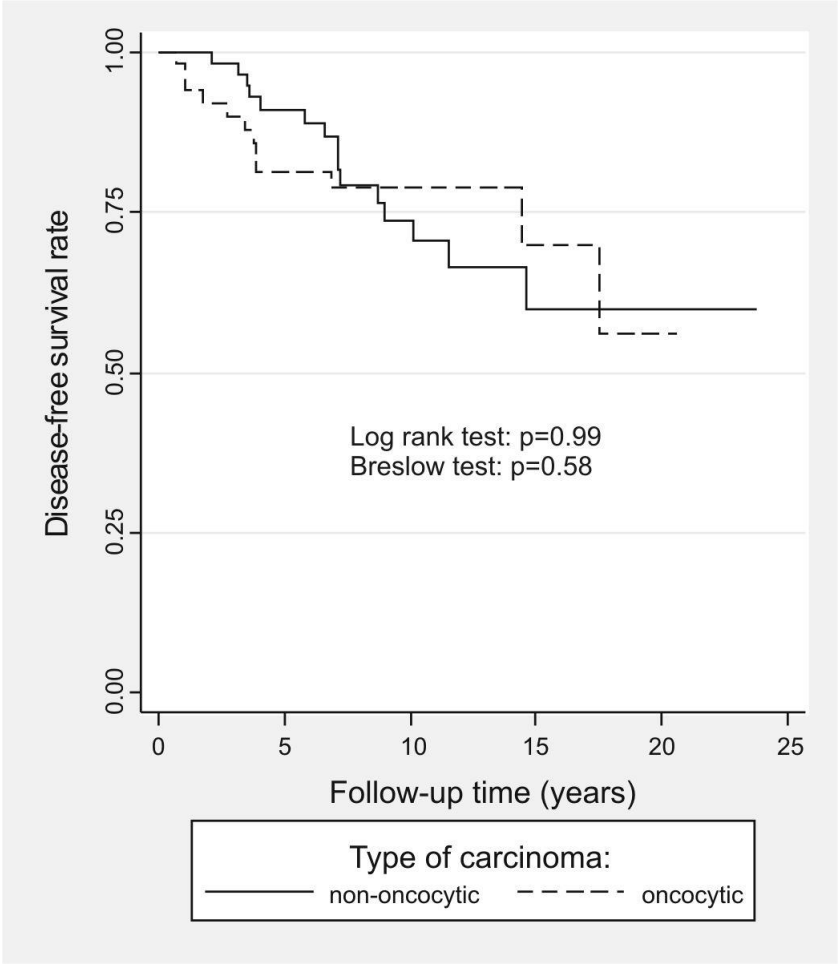
Oncocytic:
- Prohibitin high (homogenous/polarized)
- NDUFS-4 neg.

I. Biological significance

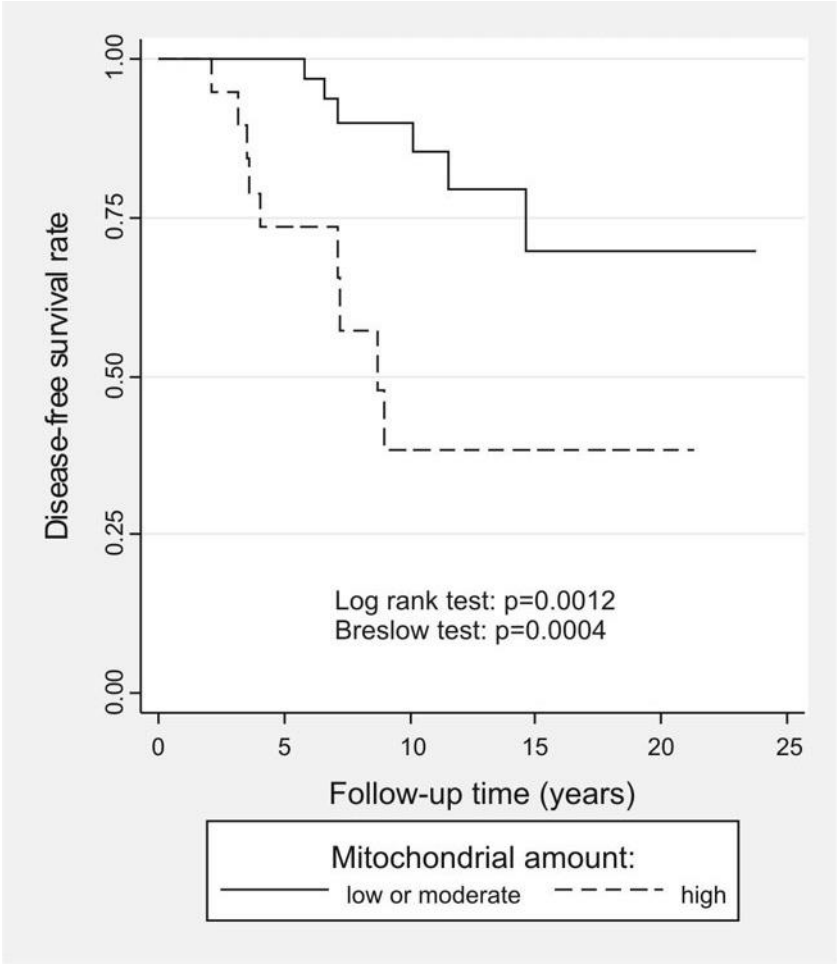
- Failure to distinguish truly oncocytic and mitochondrion-rich non-oncocytic lesions => huge gap in our knowledge

Prognostic significance

All carcinomas



Non-oncocytic carcinomas

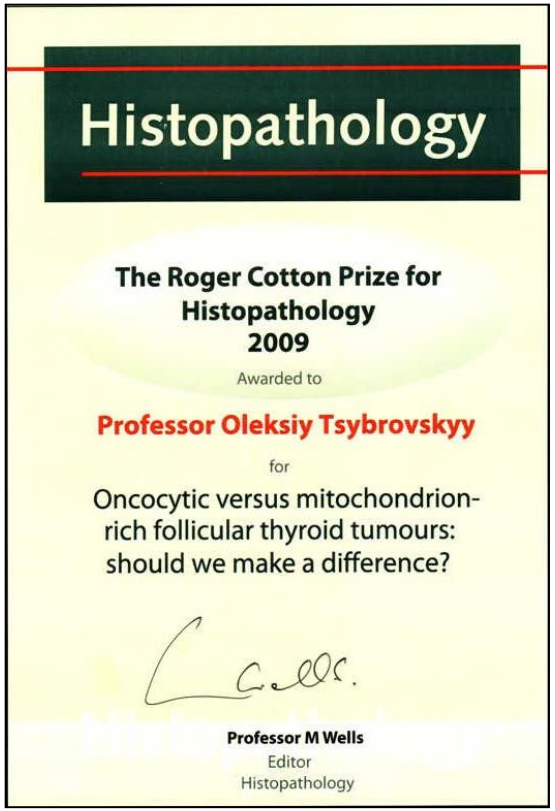


VINCENT ACADEMY OF PATHOLOGY

Histopathology 2009, **55**, 665–682. DOI: 10.1111/j.1365-2559.2009.03441.x

Oncocytic versus mitochondrion-rich follicular thyroid tumours: should we make a difference?

Oleksiy Tsybrovskyy & Martina Rößmann-Tsybrovskyy
Institute of Pathology, Medical University of Graz, Graz, Austria



II. Pathomorphological significance

- Nuclear atypia, incl. PTC-like features -> problematic if oncocytic change is under-recognized:
  - False-positive FNA reports
  - Over-diagnosis of NIFT-P
- Blurred morphology FTC vs. PTC (nuclear and architectural)
- Reduced expression of TPO in benign oncocytic lesions
- Reduced expression of PTC-markers (galectin 3, HBME-1, CK19) in case of oncocytic PTC
- False-positive IHC results (depending on detection kit)

False-positive IHC results

Applied Immunohistochemistry 5(4): 223–228, 1997

Antibodies to Cytokeratin 14 Specifically Identify Oncocytes (Hürthle Cells) in Thyroid Lesions and Tumors

Giuseppe Santeusanio, M.D., Valeria D'Alfonso, M.D., Edmondo Iafrate, M.D., Alfredo Colantoni, M.T., Fabrizio Liberati, M.D., Spagnoli Luigi Giusto, M.D., and Allen M. Gown, M.D.

"The antibody to CK14 specifically immunostained neoplastic and non-neoplastic lesions composed of Hürthle cells…"

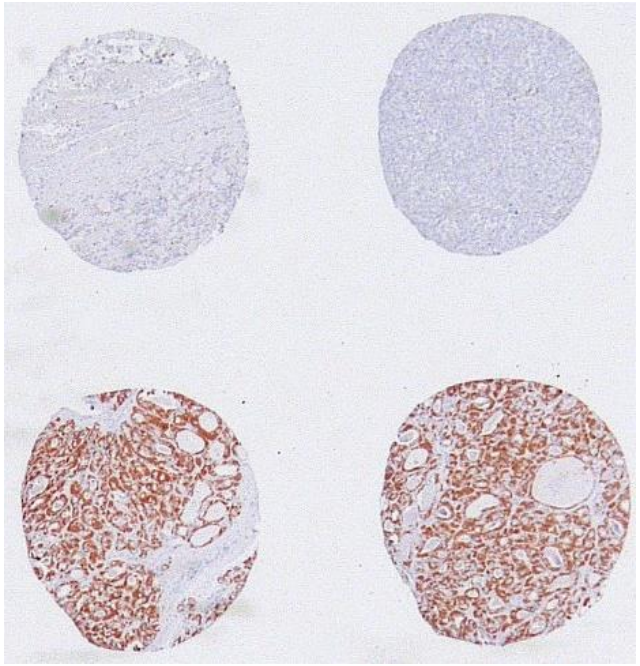
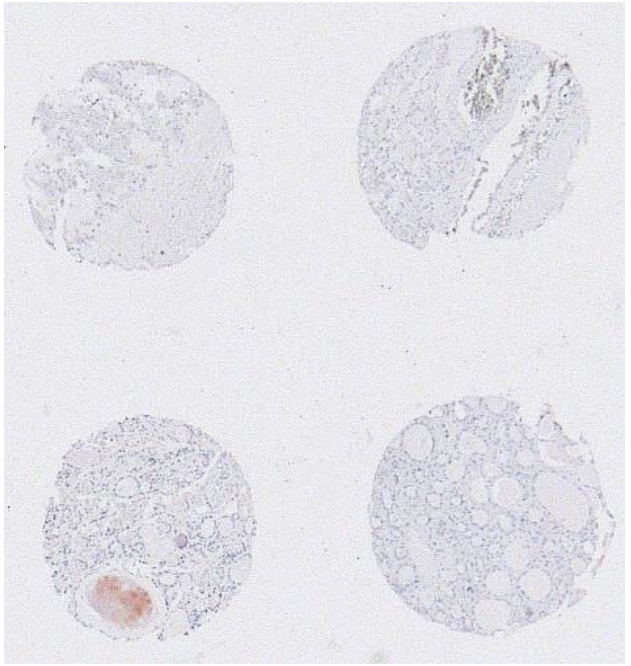
Histopathology 2001, **39**, 455–462

Cytokeratin 14 immunoreactivity distinguishes oncocytic tumour from its renal mimics: an immunohistochemical study of 63 cases

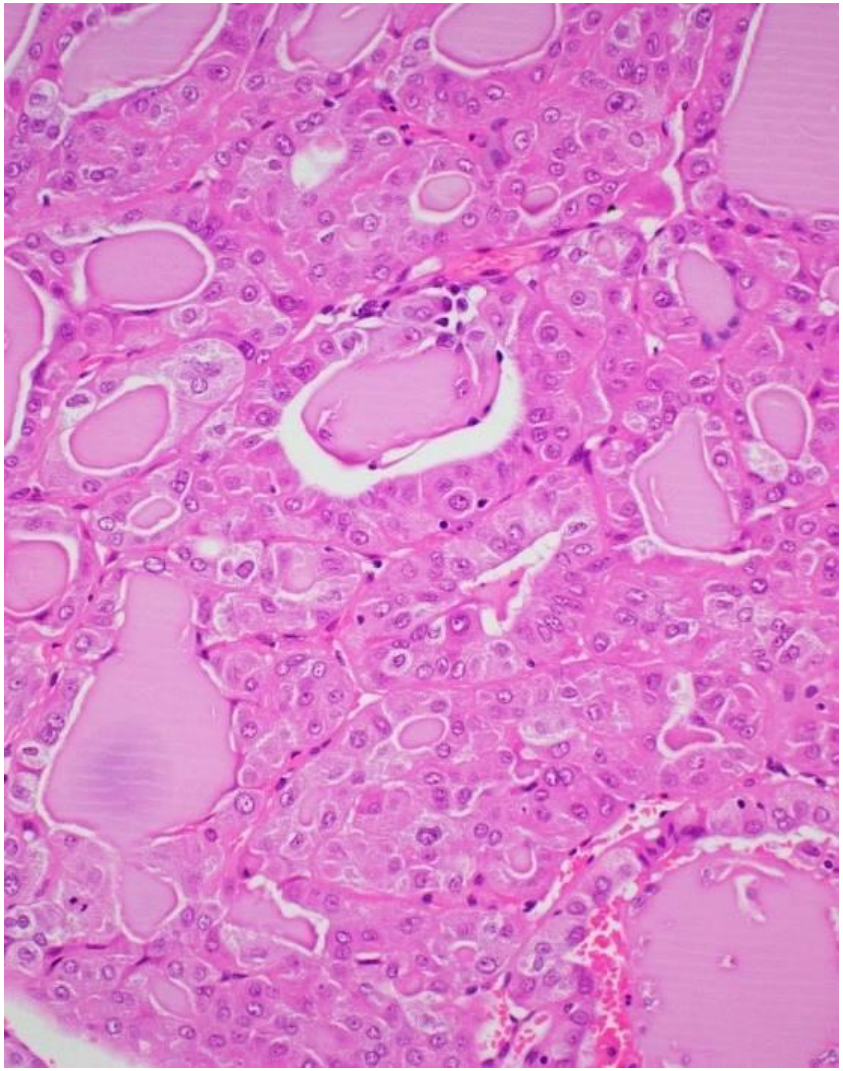
P G Chu & L M Weiss
Division of Pathology, City of Hope National Medical Center, Duarte, CA, USA

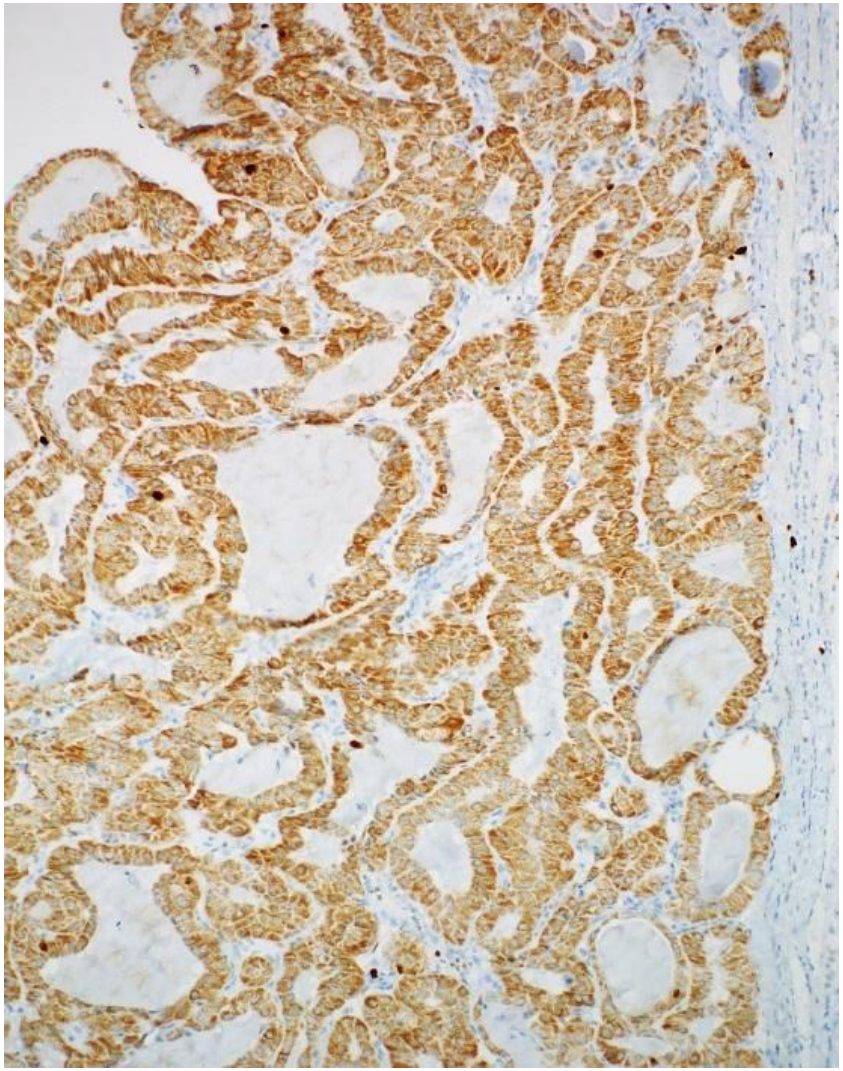
"The homogenous, cytoplasmic and granular CK14 immunoreactivity is sensitive and specific for oncocytic tumors…"

False-positive IHC results: CK14

	Ventana iView	DAKO polymer
Non-oncocytic		
Oncocytic		

MIB-1 staining with Ventana iView



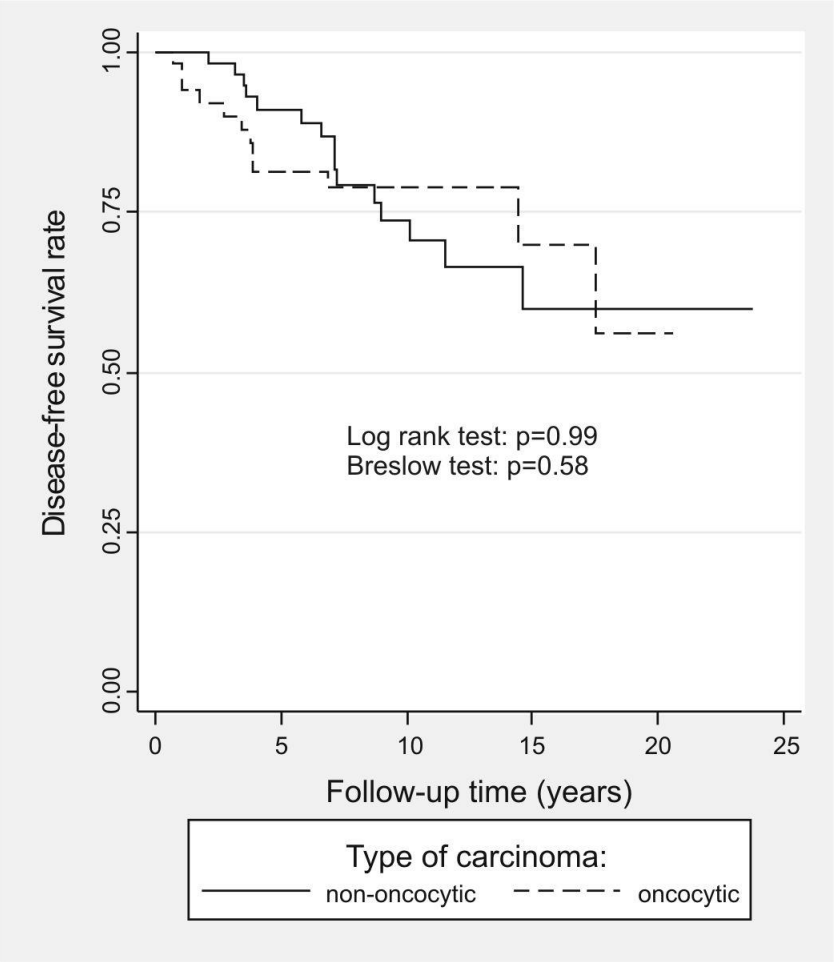


III. Clinical significance

- High glucose consume => strongly positive on FDG-PET scan (mimicking primary or metastatic malignancy, often discovered incidentally while staging cancers of breast, lung etc.)
- Loss of function => low iodine uptake
  - "Cold" appearance on scintigraphy
  - Resistance to radioiodine treatment
  - Poor sensitivity of iodine scans in follow-up (FDG-PET is much better)

If we put the things together…

All carcinomas



+

"Oncocytic carcinomas are resistant to radioiodine treatment"

⇓

In order to equate the radioiodine resistance, oncocytic carcinomas must be actually less biologically aggressive than non-oncocytic ones

Conclusions

Oncocytic = mitochondrion-rich, but not only this!!!
+ abberant organelle distribution + complex I defect

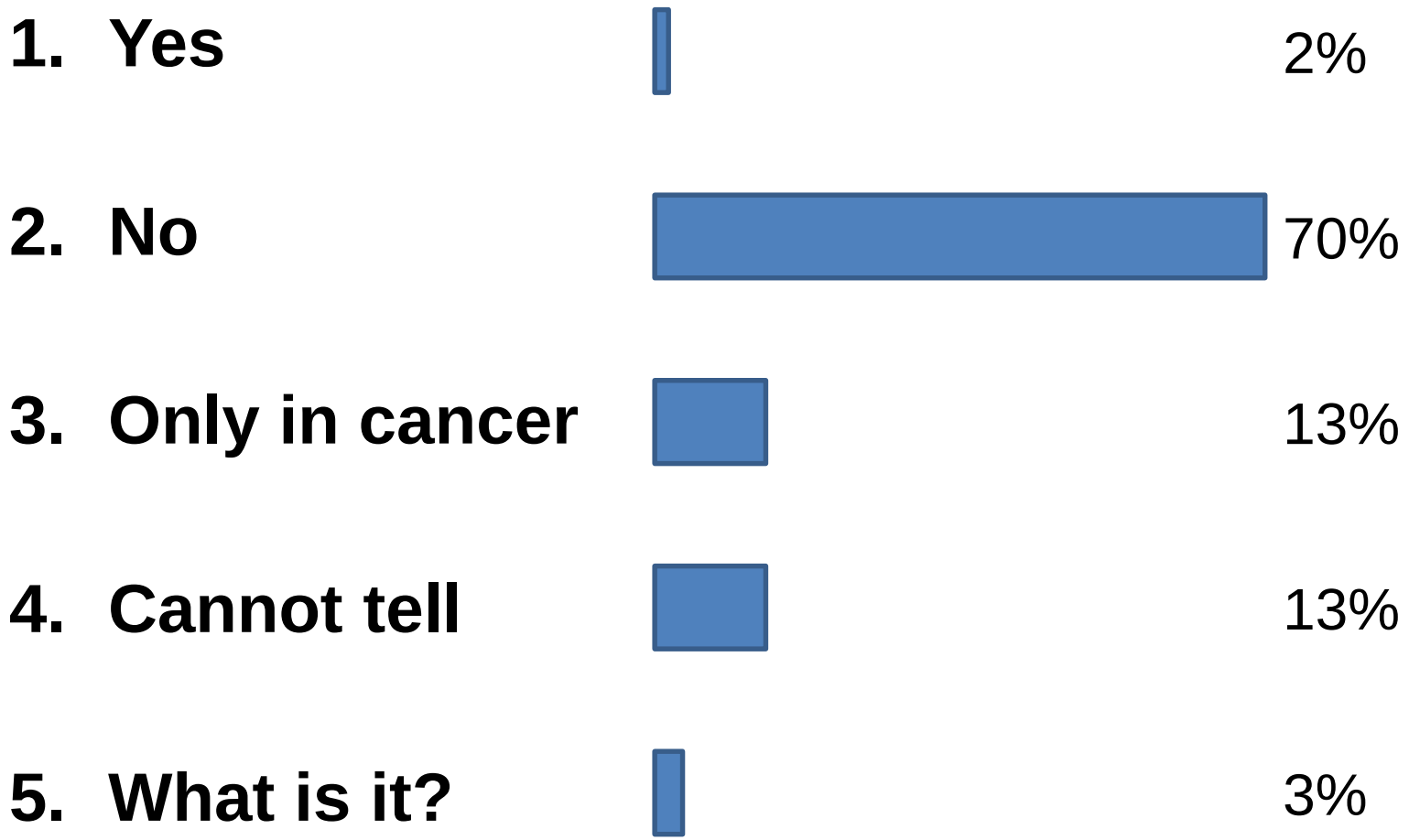
Mitochondrion-rich ≠ oncocytic!!!

Other mechanisms
(possibly related to
neoplasia/malignancy)

Oncocytic change
(a kind of cell damage,
aging or impairment)

H&E is not reliable in diagnosing subtle oncocytic change and assesing mitochondrial amount in non-oncocytes

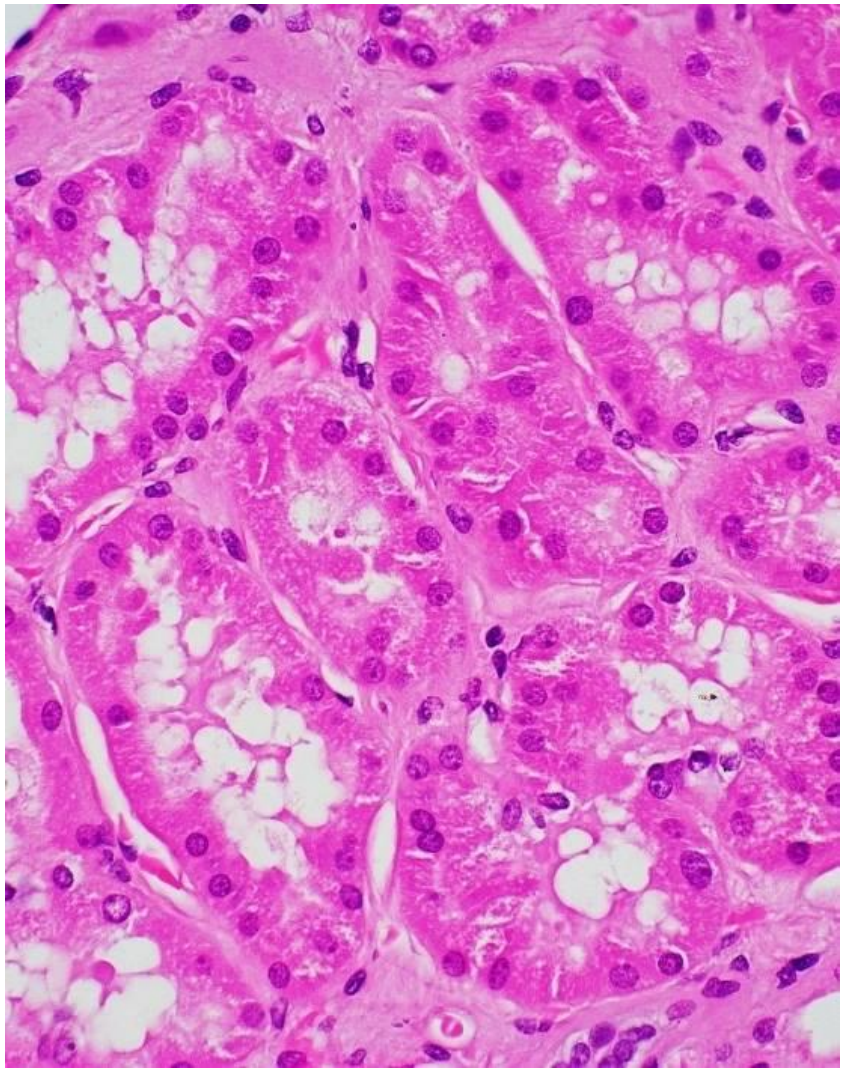
Are oncocytes in the thyroid biologically more aggressive than non-oncocytes?



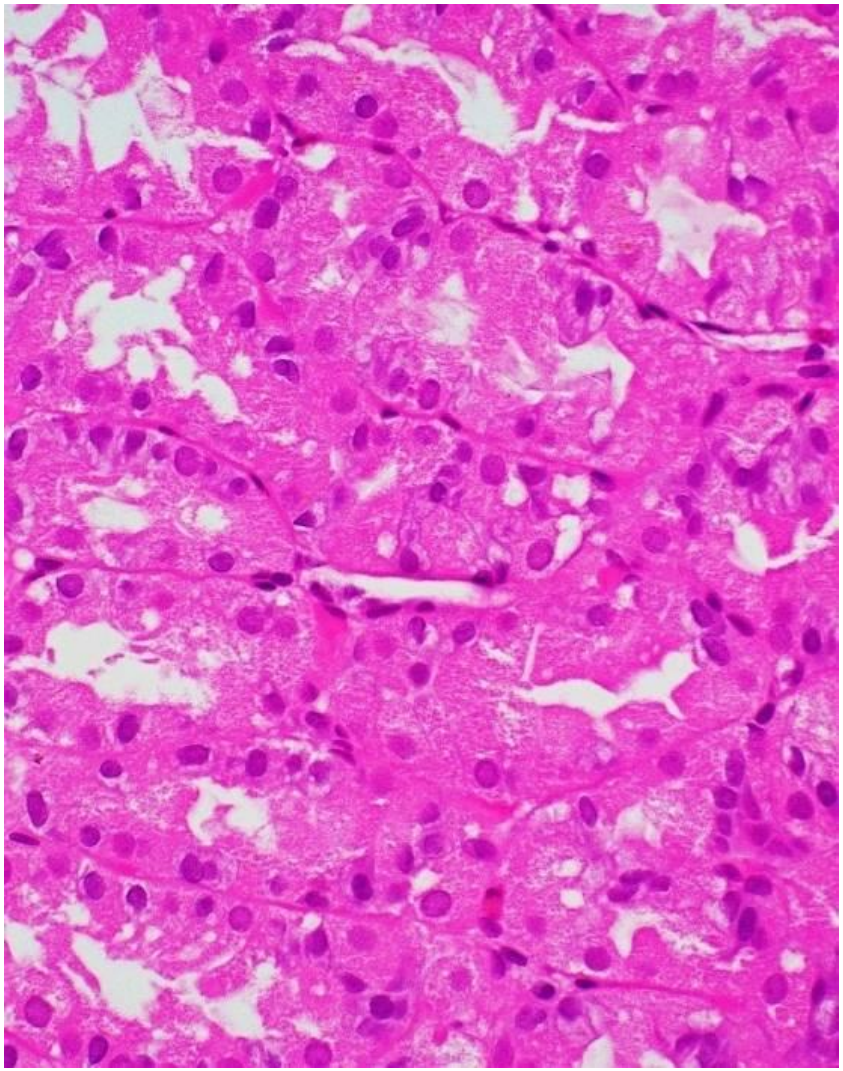
Quiz: which one is oncocytic?

VINCENT ACADEMY OF PATHOLOGY

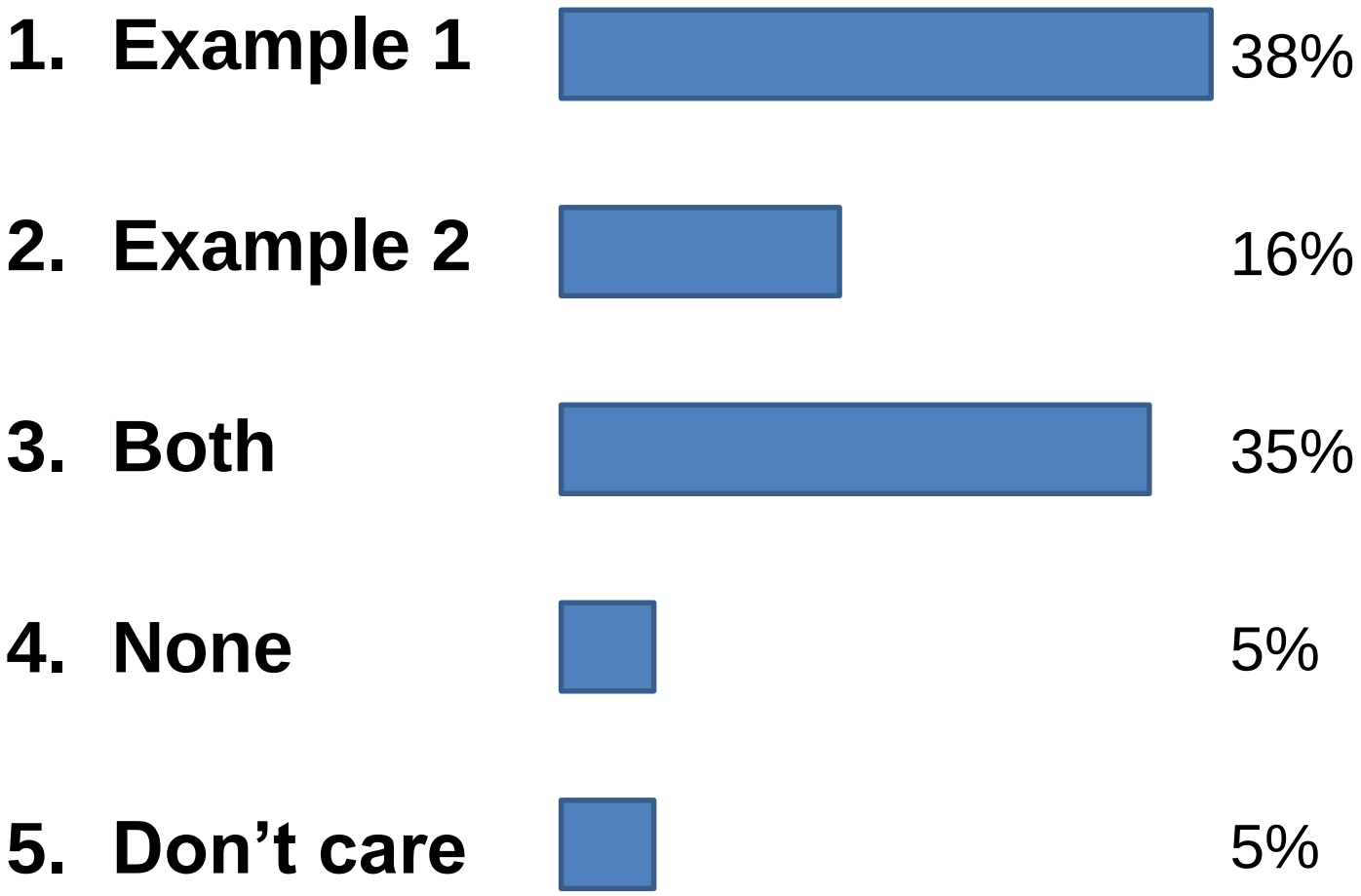
Example 1



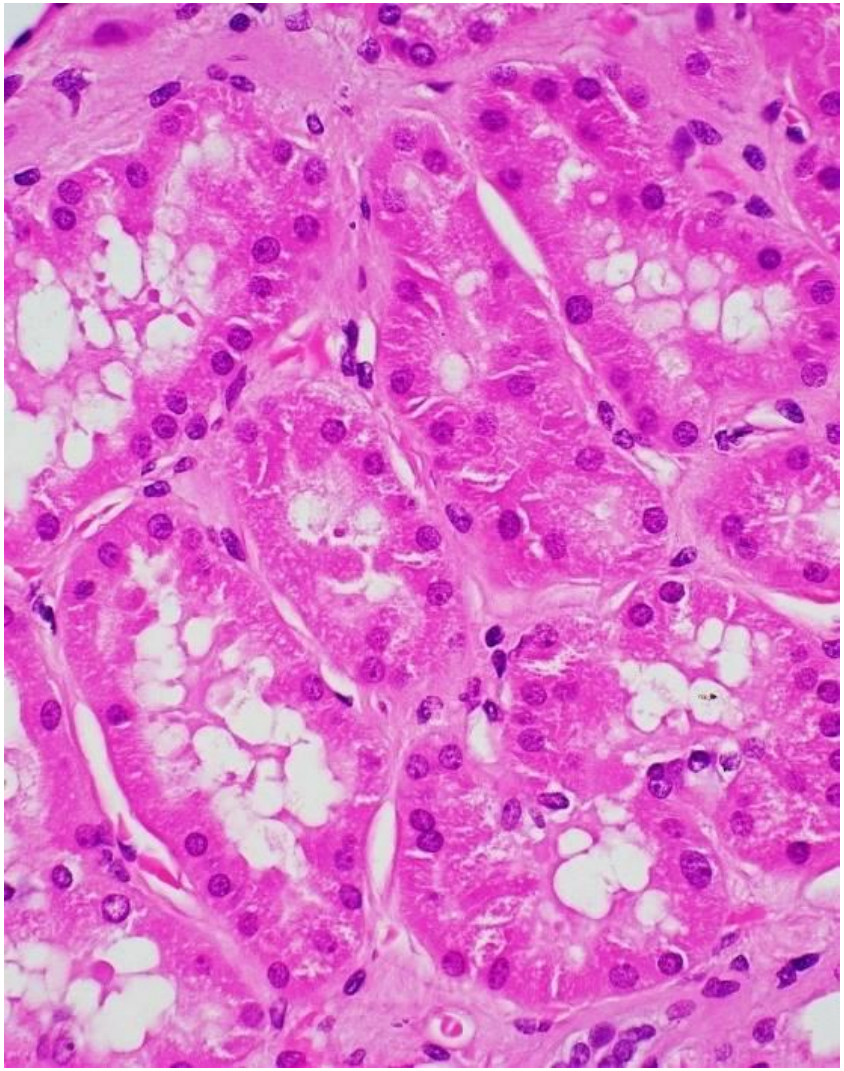
Example 2

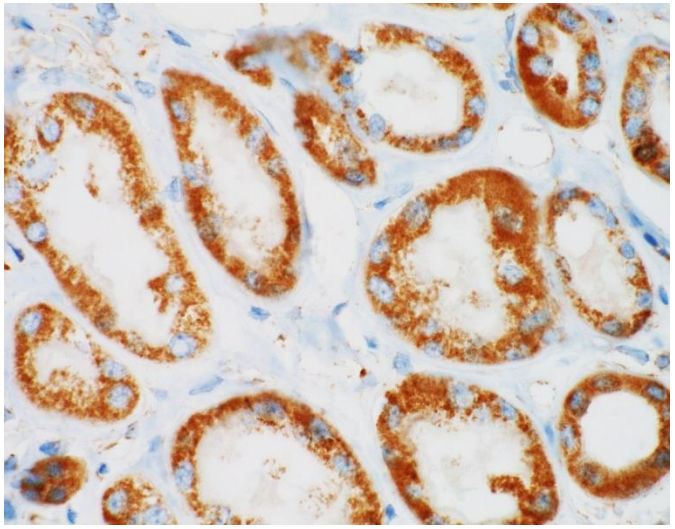


Which example is oncocytic?

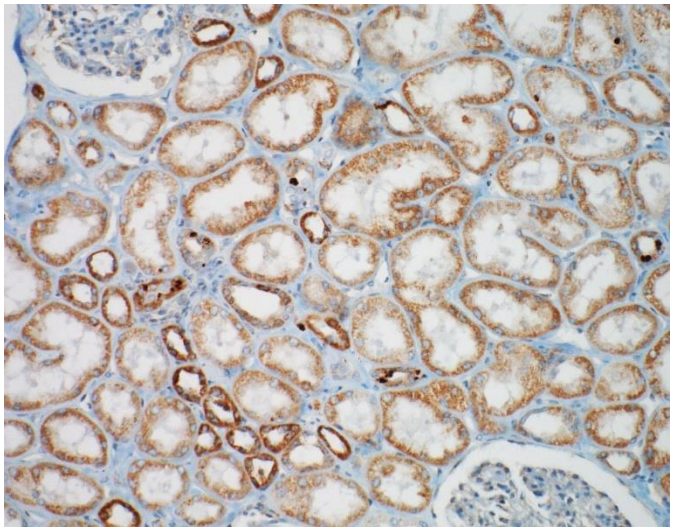


Example 1 is… normal renal parenchyma





Prohibitin



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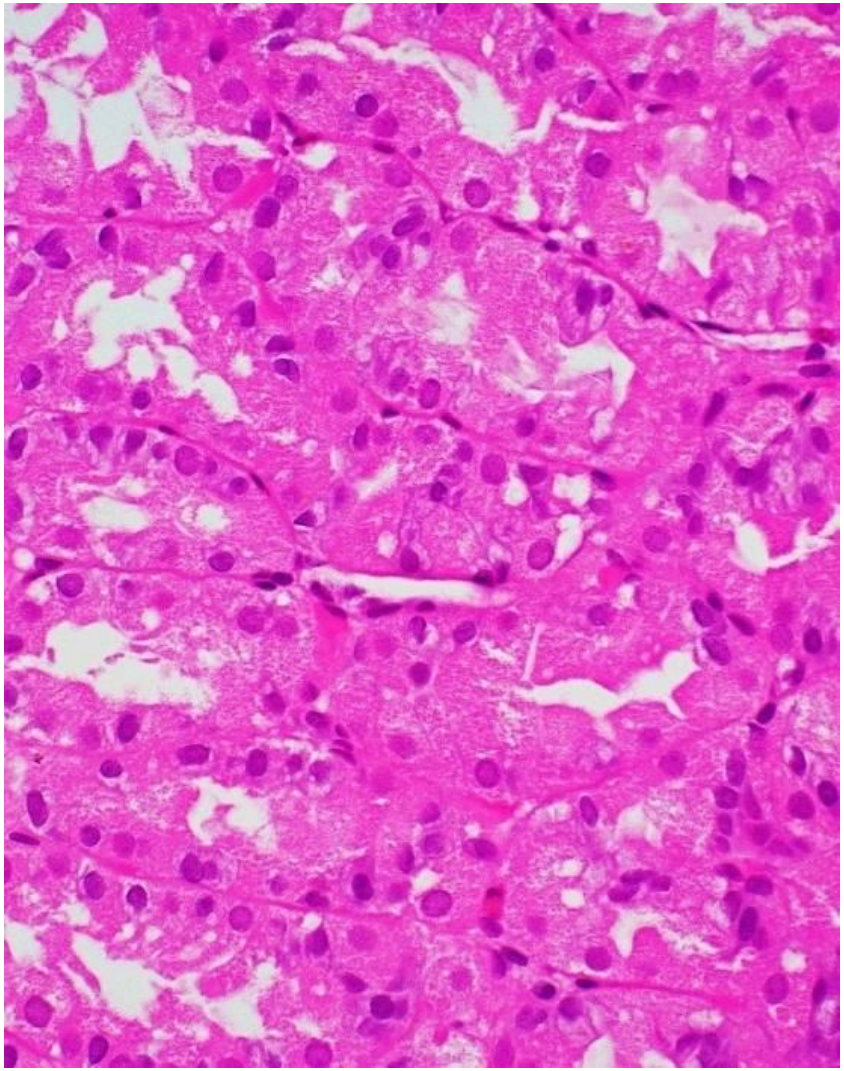
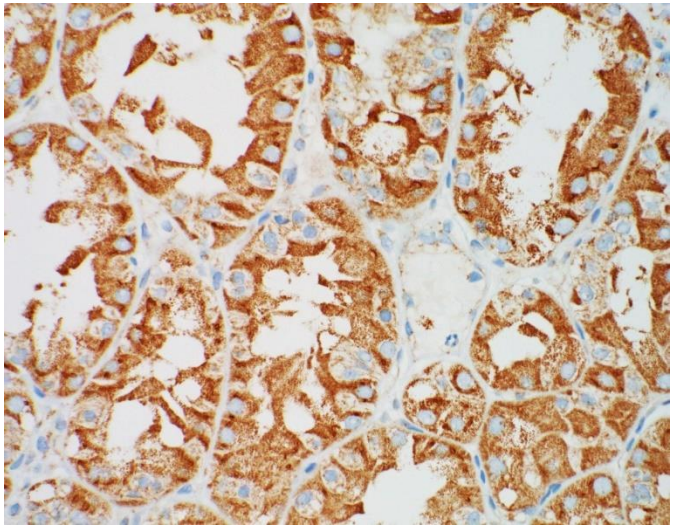
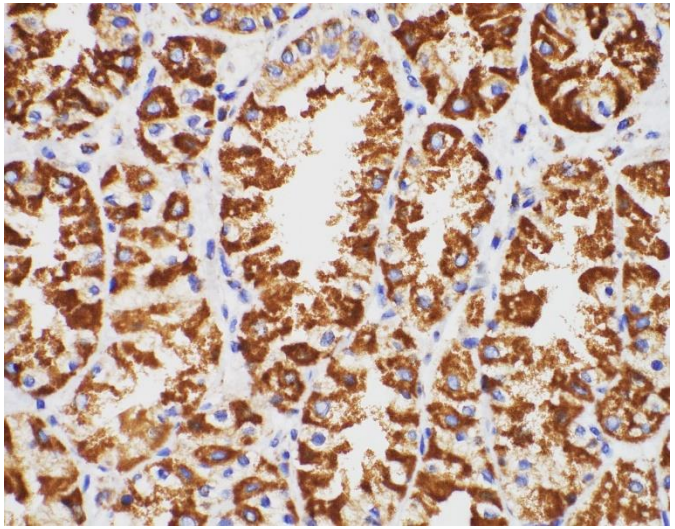
Example 2 is… normal gastric mucosa under PPI





Prohibitin



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