Importância da genética molecular no diagnóstico, prognóstico e selecção terapêutica dos tumores da tireoide

Valencia, 27 de Abril de 2012

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Faculdade de Medicina da Universidade do Porto/Centro Hospitalar S. João e IPATIMUP (Instituto de Patologia e Imunologia Molecular da Universidade do Porto)

The importance of understanding

The primary occurrence of BRAF(V600E) is a rare clonal event in papillary thyroid carcinoma

Guerra A et al. J Clin Endocrinol Metab 97:517, 2012

- Using a more sensitive approach than Sanger sequencing (pyrosequencing) it was shown that only a fraction of tumor cells (44.7% - 5%) displayed the BRAF V600E mutation

- BRAF mutated tumours are genetically heterogenous

- The BRAF V600E mutation in PTCs occurs as a late clonal event during tumor development.

- Benign *vs* Malignant *vs* Borderline (Uncertain malignant potential – UMP)
- Lobectomy *vs* Lobectomy plus isthmectomy *vs* Total thyroidectomy
- Radioactive iodine: Yes or No?
- Targeted therapies: When and Which one(s)?

Cytology
Histology
+
Molecular
Pathology

THYROID CARCINOMAS

Follicular carcinoma
Papillary carcinoma
(Hürthle cell carcinoma)

Medullary carcinoma
Poorly differentiated ca

Undifferentiated ca

WHO book on Endocrine Tumours, 2nd edition, Zurich, 1986

WHO book on Endocrine Tumours, 3rd edition, 2004

Major problems in thyroid oncology

- **Separation of follicular cell from C-cell derived tumours**
- **Risk stratification in pre-malignant lesions**

  Nodular C cell hyperplasia versus micro medullary ca
  Incipient foci of malignancy in an otherwise benign lesion

- **Diagnosis of malignancy**
- **Prognosis**
- **Therapy selection**

Questions to be made whenever facing a strange lesion in the thyroid

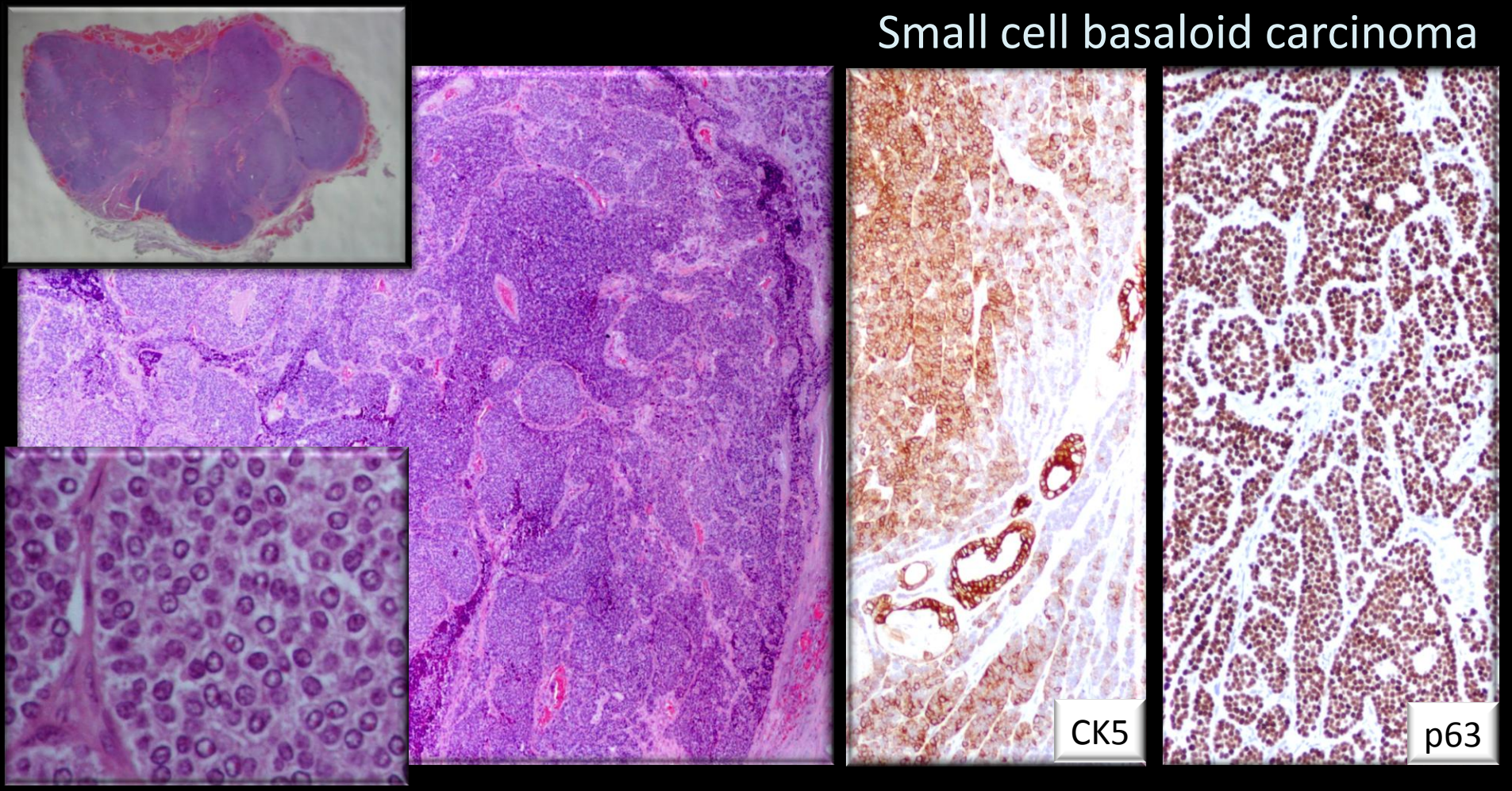
Is it a primary thyroid tumour?

If yes, is it made of follicular or C-cells?

Immunohistochemistry is mandatory: TG and calcitonin (and, if necessary, TTF1)

TTF1 positive rare flowers: Calcitonin free medullary carcinoma, Thyroglobulin negative adenoma and carcinoma, Cribriform morular carcinoma

TTF1 negative rare flowers: SETTLE, CASTLE, Small cell basaloid tumours



Cruz et al. Int J Surg Pathol, 2011 & Eloy C et al. Int J Surg Pathol, 2012

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- Diagnosis of malignancy
- Prognosis
- Therapy selection

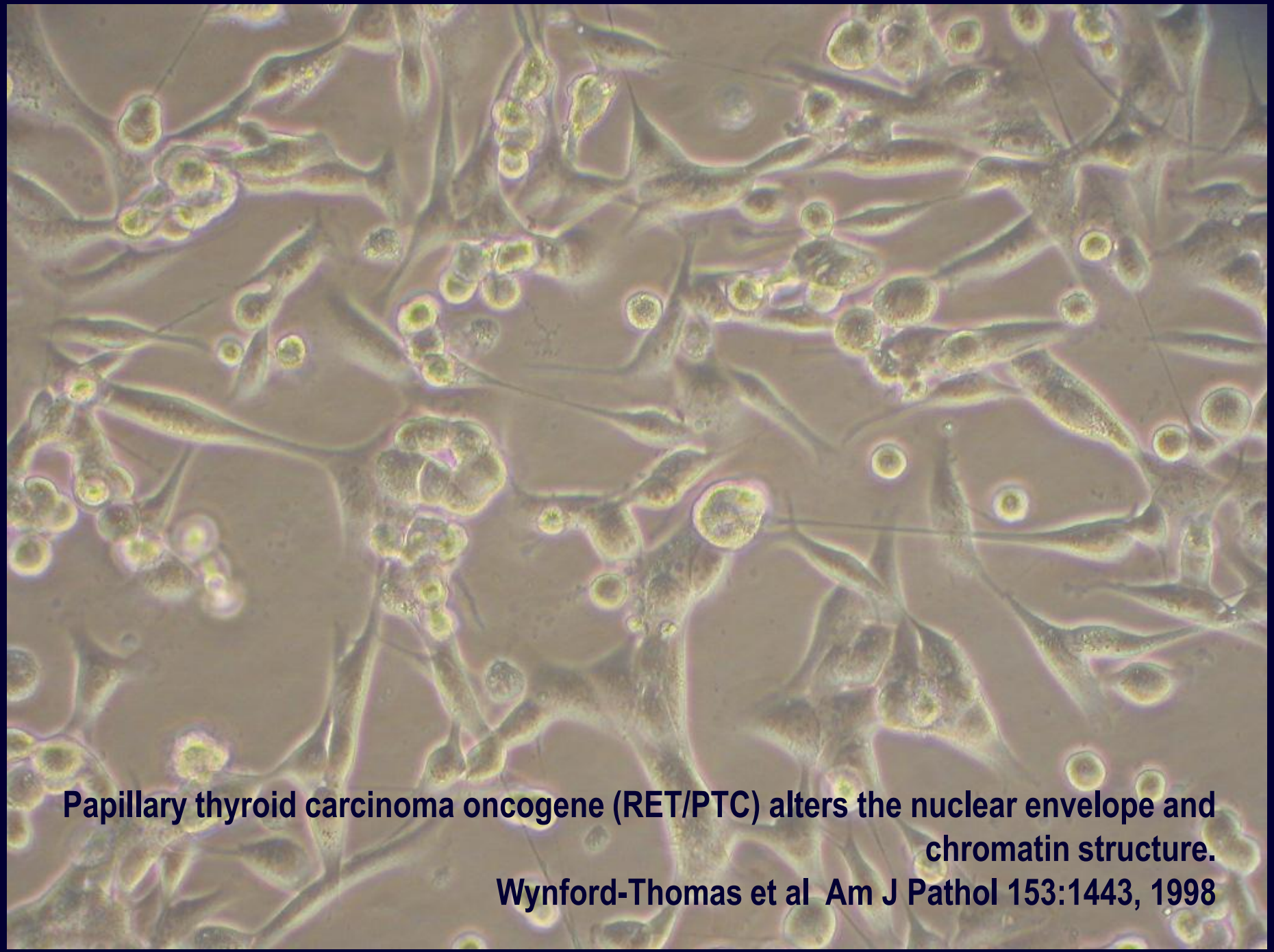
Irradiation
Other factors

No chromosomal instability
Diploidy; Near diploidy

Follicular cell → Conventional Papillary ca

RET/PTC (mainly RET/PTC1) and TRK rearrangements
BRAF mutations (V600E)
C-MET and EGF-R overexpression
mTOR overexpression
TGFβ and STAT 3 alterations
E-cadherin downregulation

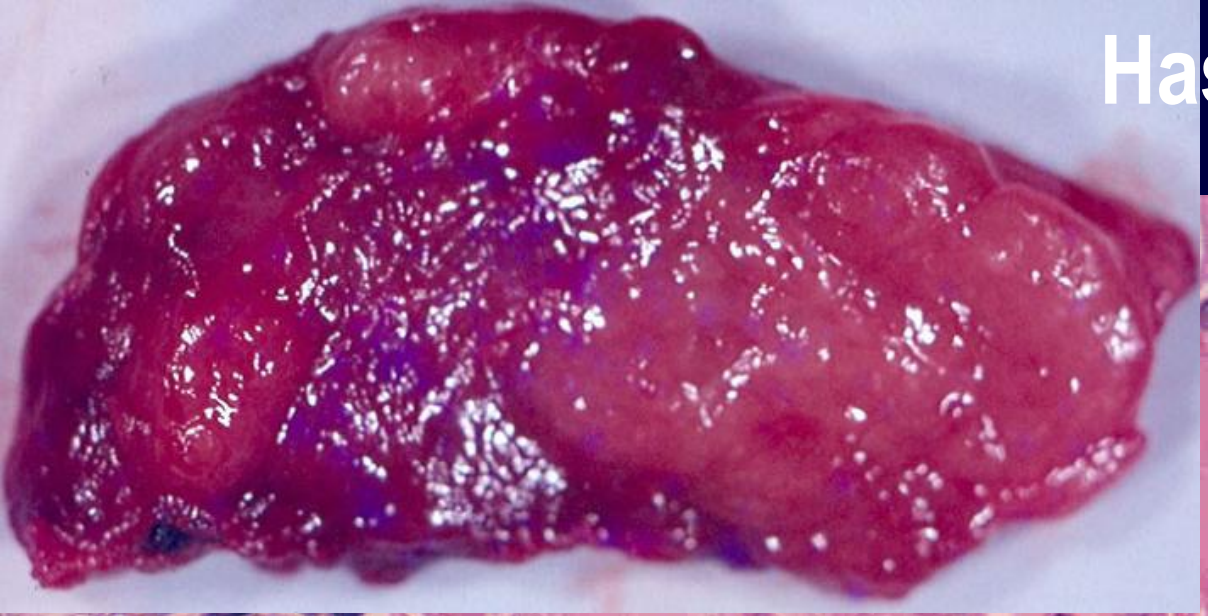
RET/PTC rearrangement



Papillary thyroid carcinoma oncogene (RET/PTC) alters the nuclear envelope and chromatin structure.

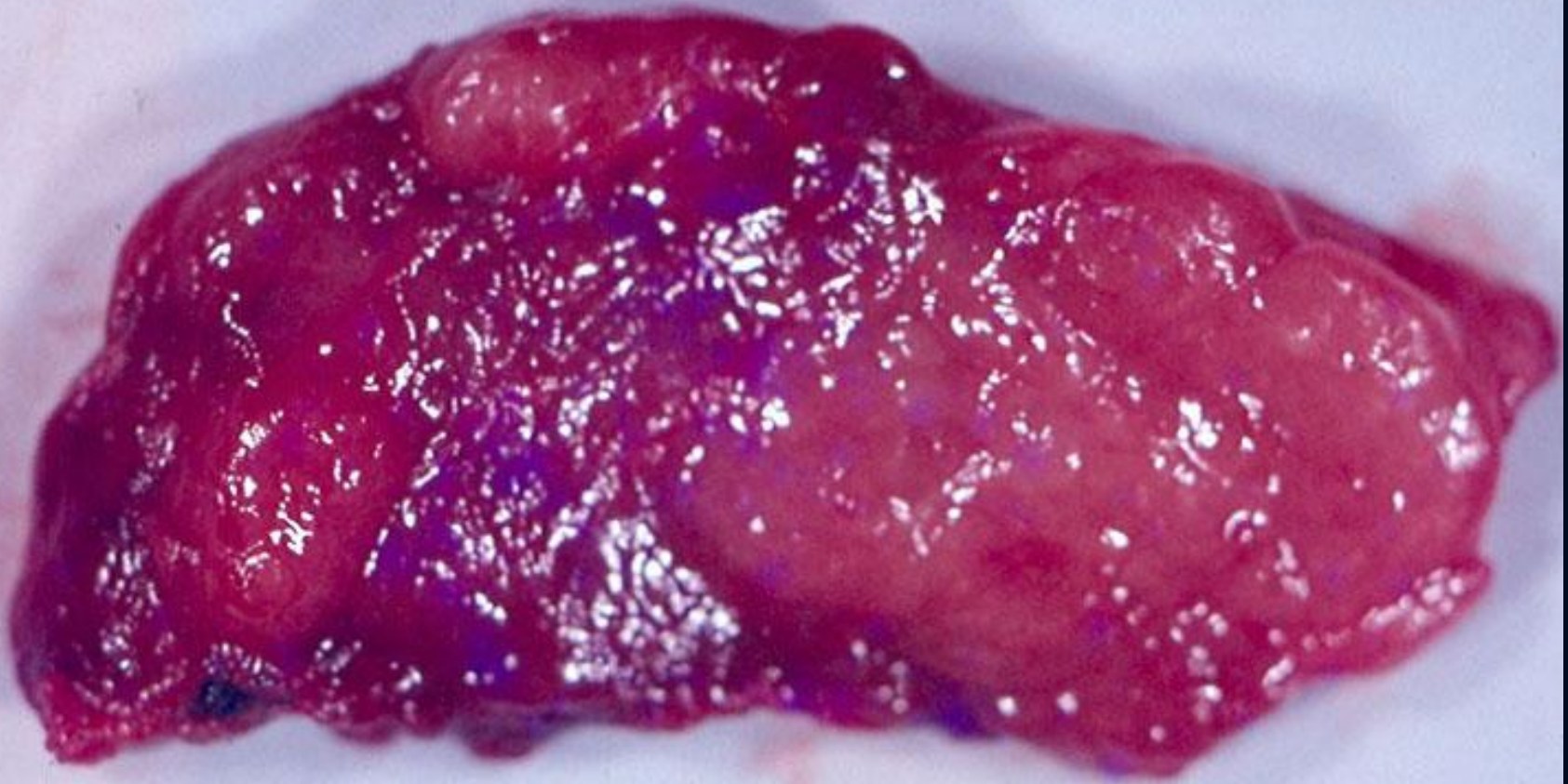
Wynford-Thomas et al Am J Pathol 153:1443, 1998

Hashimoto thyroiditis



RET/PTC positive

Hashimoto thyroiditis



68% RET/PTC positivity

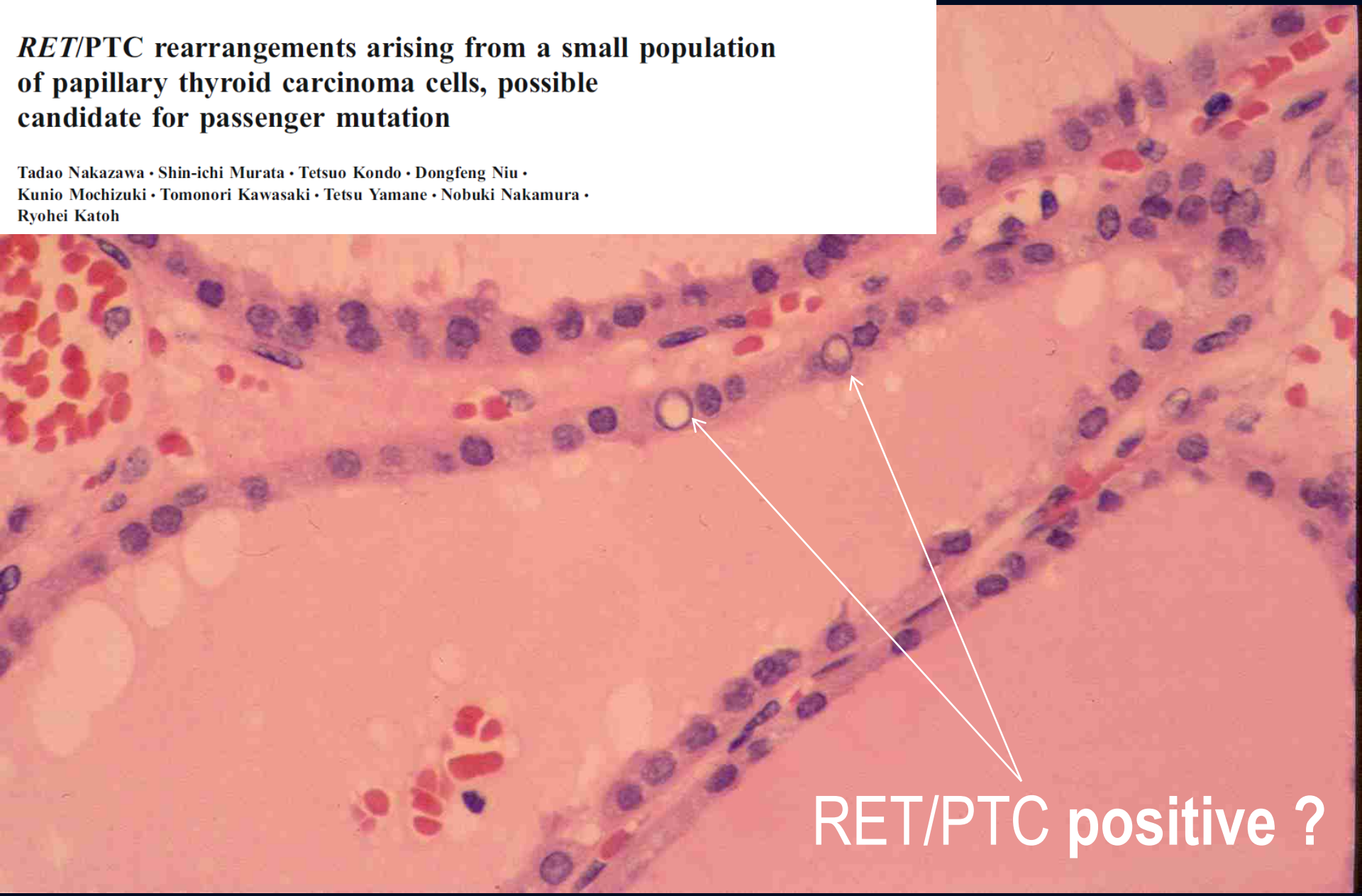
Rhoden KJ. J Clin Endocrinol Metab 91:2414-23, 2006

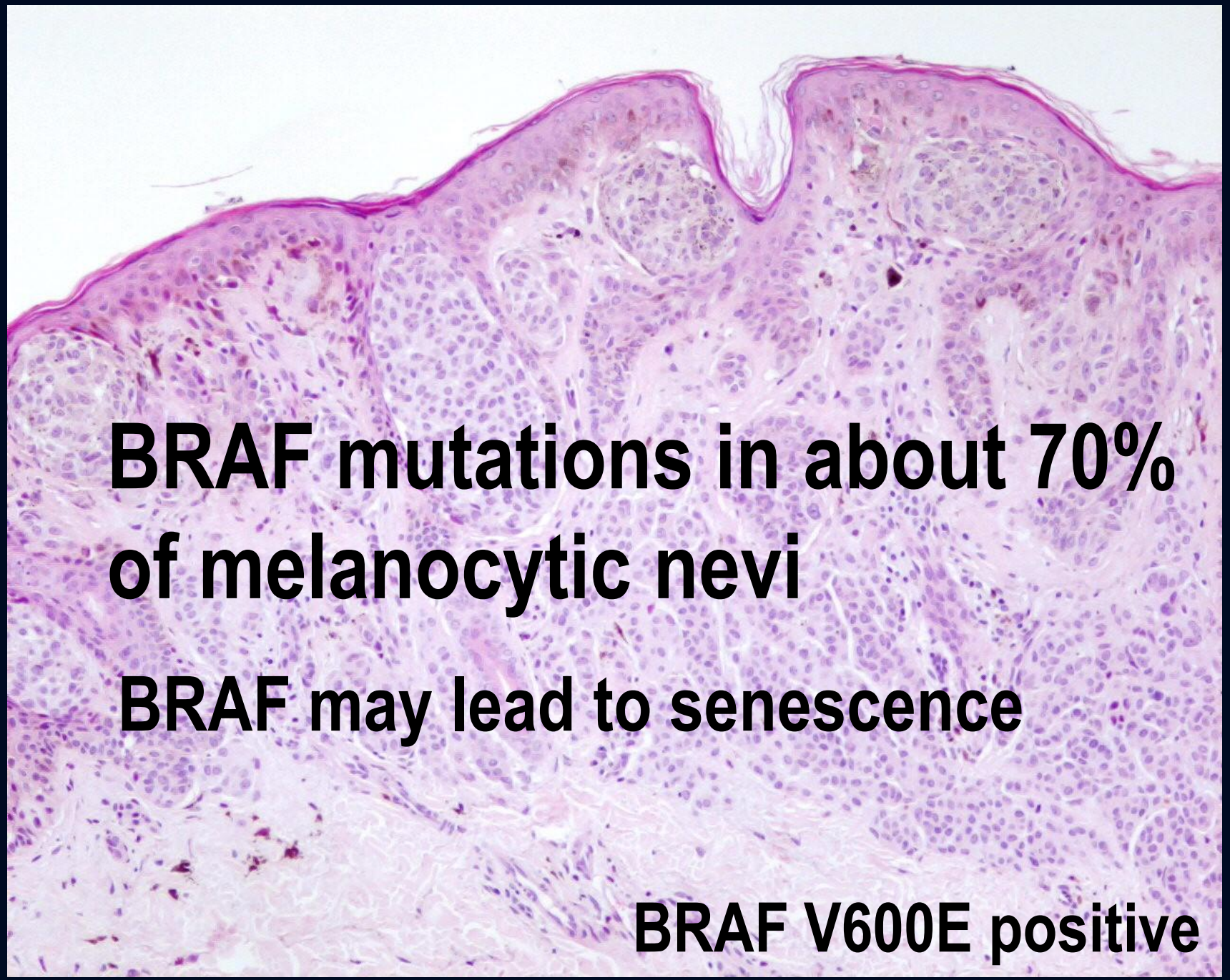
Virchows Arch (2009) 455:35–41
DOI 10.1007/s00428-009-0789-8

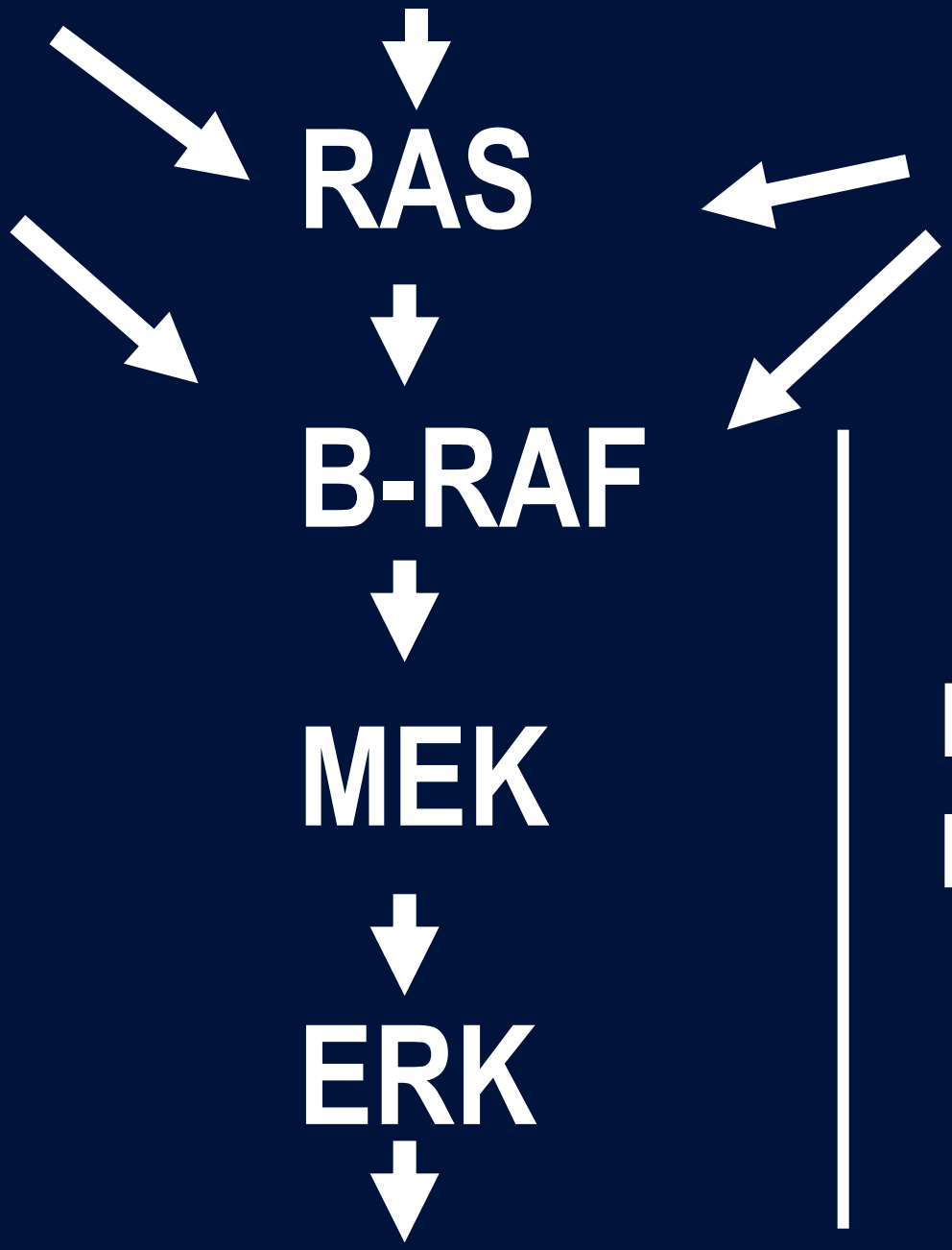
ORIGINAL ARTICLE

RET/PTC rearrangements arising from a small population of papillary thyroid carcinoma cells, possible candidate for passenger mutation

Tadao Nakazawa · Shin-ichi Murata · Tetsuo Kondo · Dongfeng Niu · Kunio Mochizuki · Tomonori Kawasaki · Tetsu Yamane · Nobuki Nakamura · Ryohei Katoh




BRAF mutations in about 70% of melanocytic nevi
BRAF may lead to senescence
BRAF V600E positive



MAPK pathway

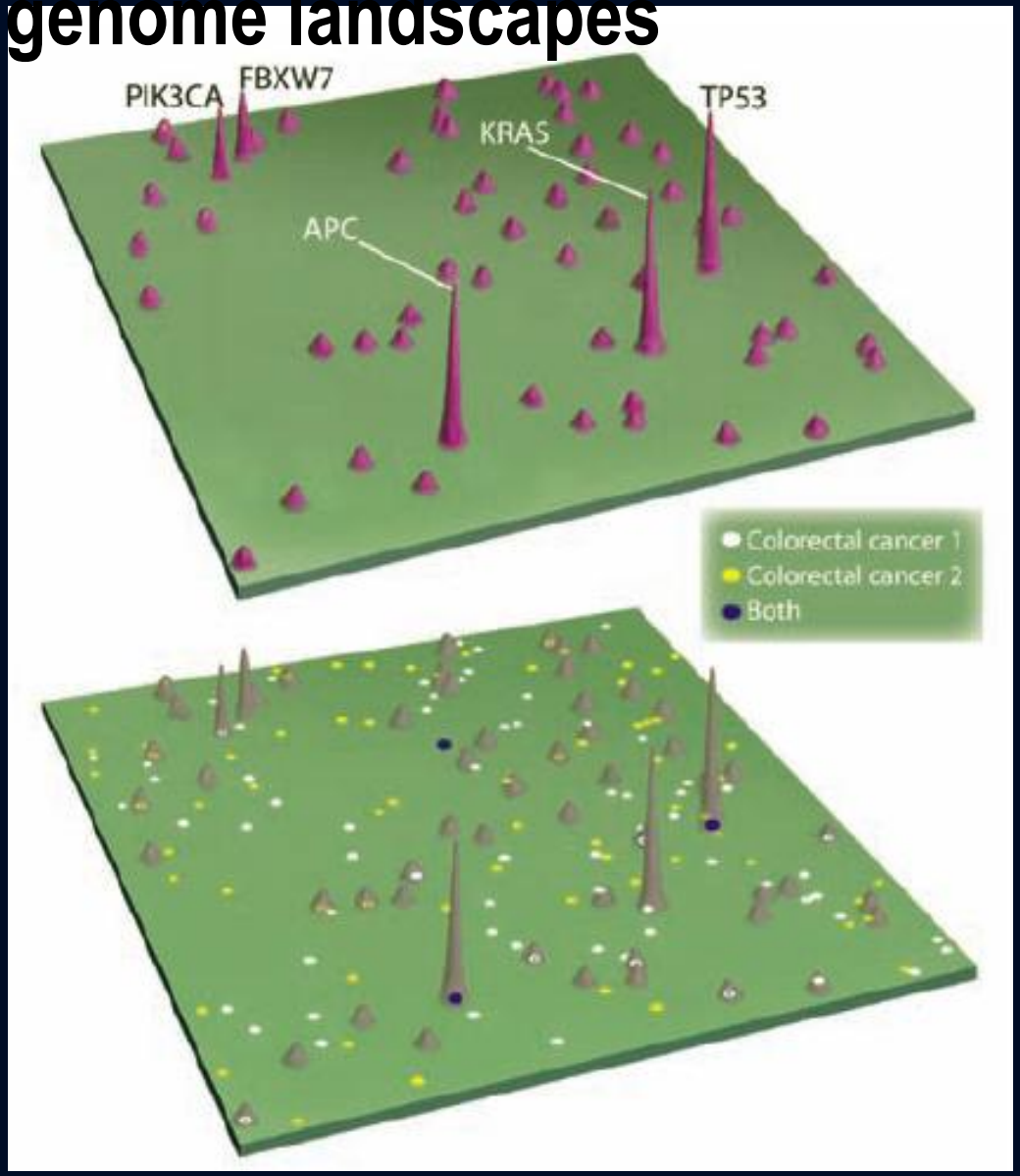
Several other pathways:
PI3K/Akt
NFkβ
mTOR
STAT3
.....

Two main paradigms for understanding human diseases

1. Core biological processes associated with a disease are driven by responses to changes in a small number of genes
2. Disease states are considered as emergent properties of molecular networks originating from a very complex interplay between constellations of changes in DNA and a broad range of factors such as diet, age, gender and exposure to environmental toxins.

Chen et al Nature 452:429, 2008
Schadt EE, Nature, September 10, 2009

Cancer genome landscapes



Driver genes & Passenger genes

Wood LD… Vogelstein B, Science 318:1108, 2007

Weaknesses of the concept of oncogene addiction

- Multiple genetic and epigenetic abnormalities (genomic instability)
- Tumour heterogeneity
- Diferences among stem cells and their progeny (differences in their intracellular circuitry)
- Differences in the host microenvironment

Felsher DW, Oncogene addiction versus oncogene amnesia: Perhaps more than just a bad habit? Cancer Res 68:3081, 2008

CANCER GENES

Oncogenes

Tumour-supressor genes

“Secondary” genetic alterations

Driver genes

Passenger genes
(Different qualities of passengers...)

Gatekeepers

Caretakers

Landscapers

Forerunners

PLUS THE ROLE PLAYED BY THE STROMA AND THE IMMUNE CELLS THAT CONTRIBUTE TO (IN)ACTIVATE GENES IN CONTEXT DEPENDENT MANNER

mTOR pathway overactivation in BRAF mutated papillary thyroid carcinoma

Faustino A,…Soares P. J Clin Endocrinol Metabol, 2012 (in press)

STAT3 Negatively Regulates Thyroid Cancer Growth In vivo (Xenografts and Transgenic Models)

…We observed that 58% of 59 human primary papillary thyroid carcinoma (PTC) cases expressed nuclear pSTAT3 in tumor cells, preferentially in association with the tumor stroma. ……STAT3 knockdown by shRNA in representative thyroid cancer cell lines (8505C, TPC-1, HTH7 and SW1736) that express high levels of pSTAT3 had no effect on in vitro growth. However, xenografted shSTAT3 cells generated larger tumors than shControl cells. Similarly, STAT3 deficiency in a murine model of BRAFV600E-induced PTC led to thyroid tumors that were more proliferative and larger than those expressing STAT3wt.

Couto JP et al. PNAS, 2012 (in press)

Chromosomal, epigenetic and microRNA-mediated inactivation of LRP1B, a modulator of the extracellular environment of thyroid cancer cells.

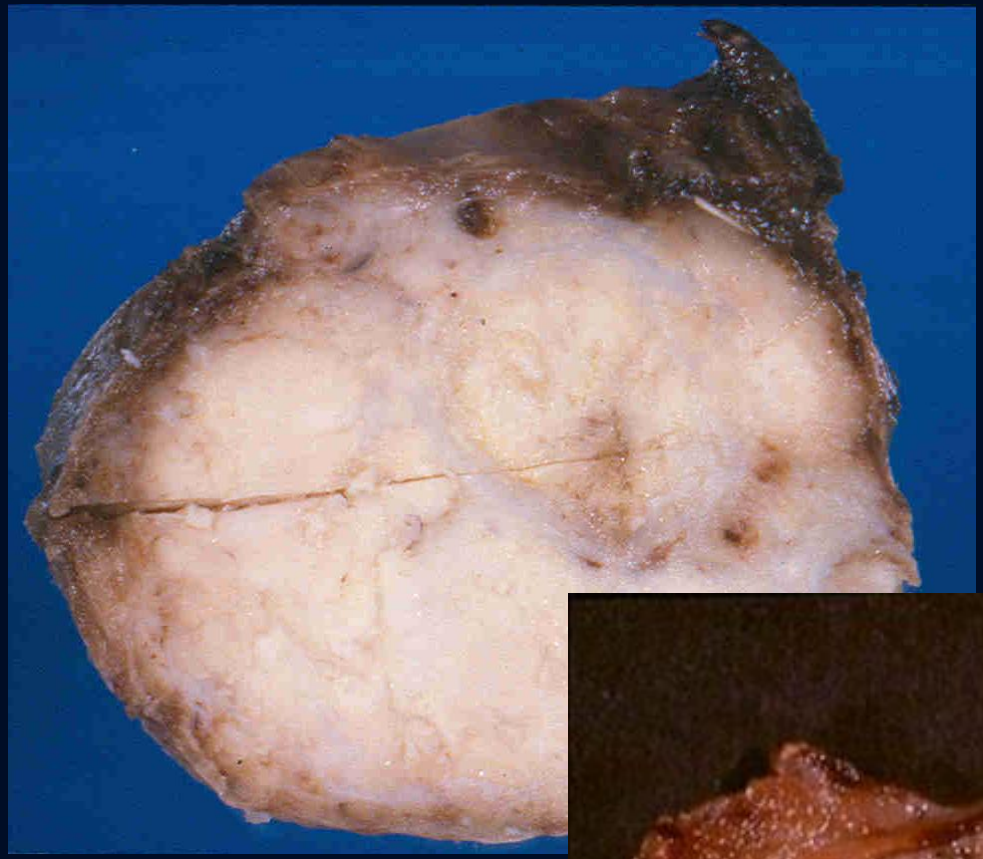
Prazeres H, Torres J, Rodrigues F, Pinto M, Pastoriza MC, Gomes D, Cameselle-Teijeiro J, Vidal A, Martins TC, Sobrinho-Simões M, Soares P.

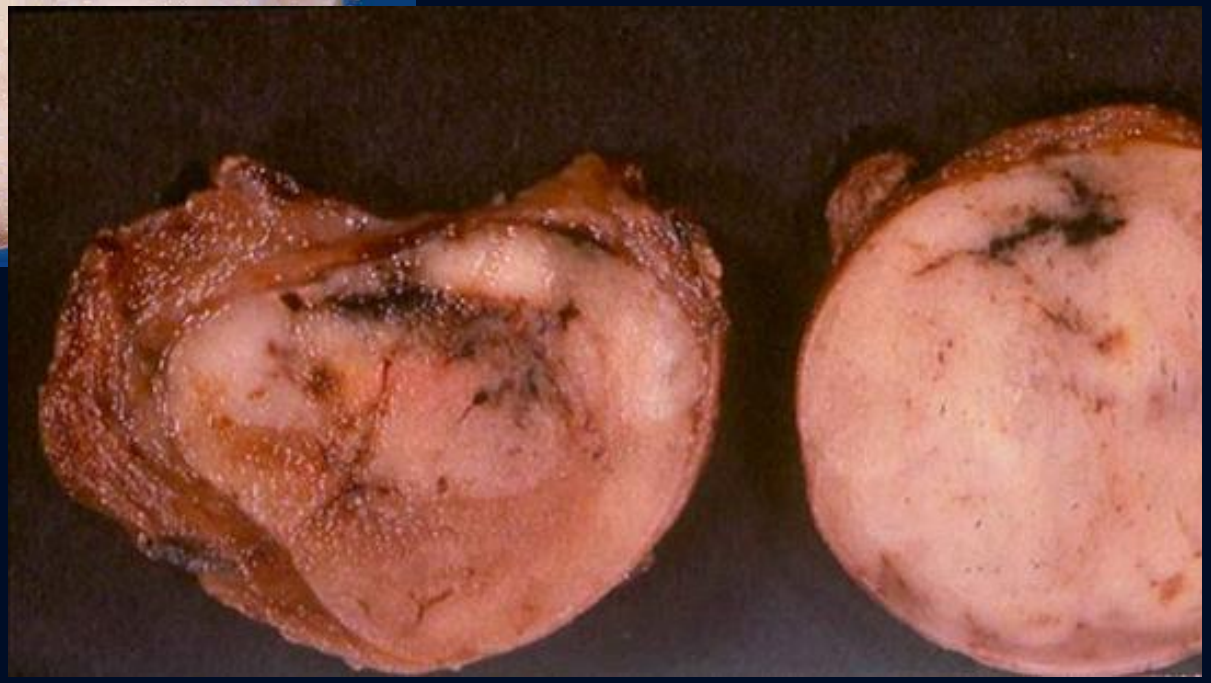
Oncogene 30:1302-17, 2011

TGF-beta/Smad pathway and BRAF mutation play different roles in circumscribed and infiltrative papillary thyroid carcinoma

Eloy C et al. Virchows Arch, 2012 (in press)

- **Transforming growth factor beta (TGF-beta)/Smad dependent pathway activity at the periphery of infiltrative PTCs is associated with epithelial-to-mesenchymal transition (EMT) and local invasion, as well as to nodal metastization**





240 cases (1978-2003) with nodal and/or distant metastases

NOT A SINGLE CASE OF:

Follicular tumour of uncertain malignant potential

Well differentiated tumour of uncertain malignant potencial

Minimally invasive follicular carcinoma without vascular invasion

Encapsulated follicular variant of PTC without invasion

Consortium IPO-IPATIMUP, 2011 (unpublished results)

IPATIMUP
Instituto de Patologia e Imunologia Molecular da Universidade do Porto

Major problems in thyroid oncology

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- Risk stratification in pre-malignant lesions
- Diagnosis of malignancy
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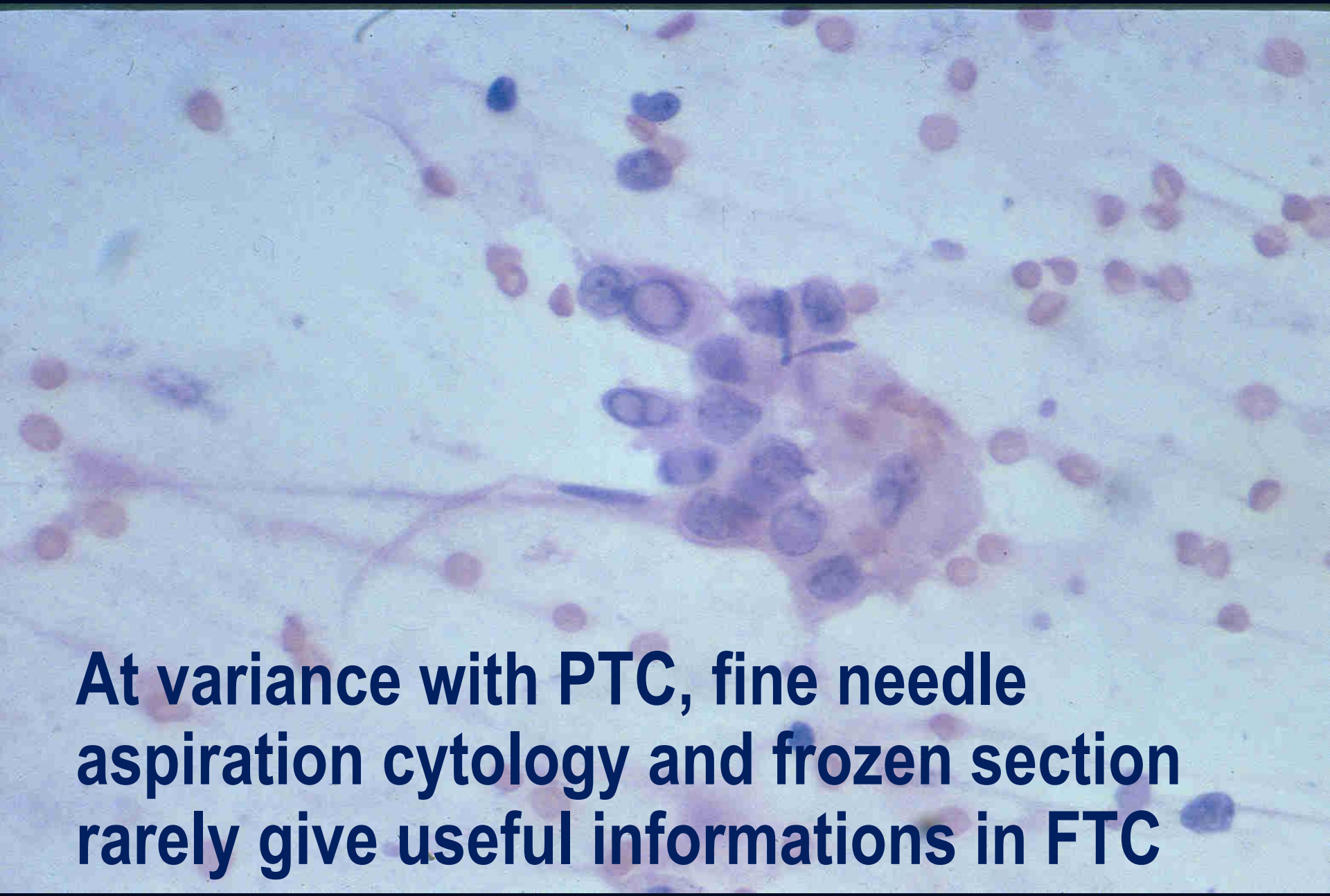
- Therapy selection



At variance with PTC, fine needle aspiration cytology and frozen section rarely give useful informations in FTC

Impact of mutational testing on the diagnosis and management of patients with cytologically indeterminate thyroid nodules: A prospective analysis of 1056 FNA samples

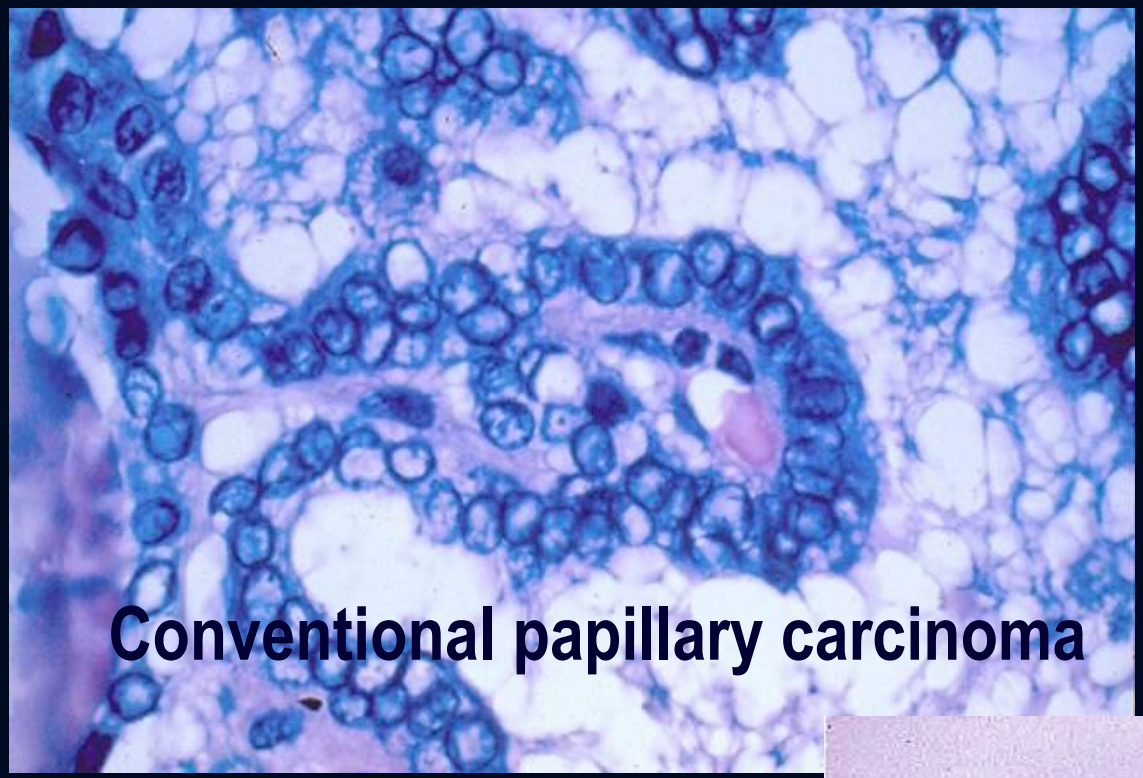
Nikiforov YE et al. J Clin Endocrinol Metab 96:3360, 2011

- Adequate material in 967 samples with indeterminate cytology: atypia of undeterminate significance, follicular neoplasm/suspicious for a follicular neoplasm and suspicious for malignant cells
- Results: 87 mutations – 19 BRAF, 62 RAS , 1 RET/PTC and 5 PAX8/PPARγ
- The detection of any mutation conferred higher risk of malignancy

The PAX8-PPARγ translocation is found in follicular tumours of uncertain malignant potential, follicular thyroid carcinoma and poorly differentiated carcinoma and is not associated with angioinvasion, capsular penetration or prognosis

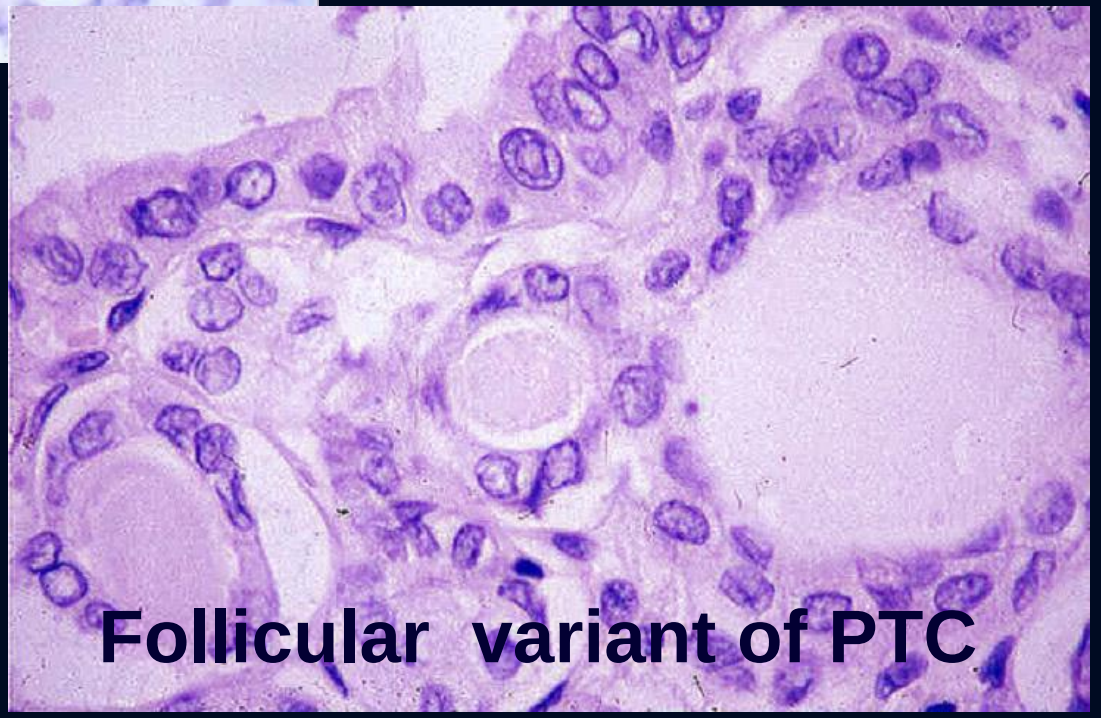
Boos LA et al. Virchows Archiv, 2012 (in press)

- **226 thyroid tumours studied by FISH**
- **PAX8-PPARg is not a reliable marker for follicular thyroid carcinoma and does not correlate with invasiveness nor with prognosis.**



Malignancy in papillary carcinoma

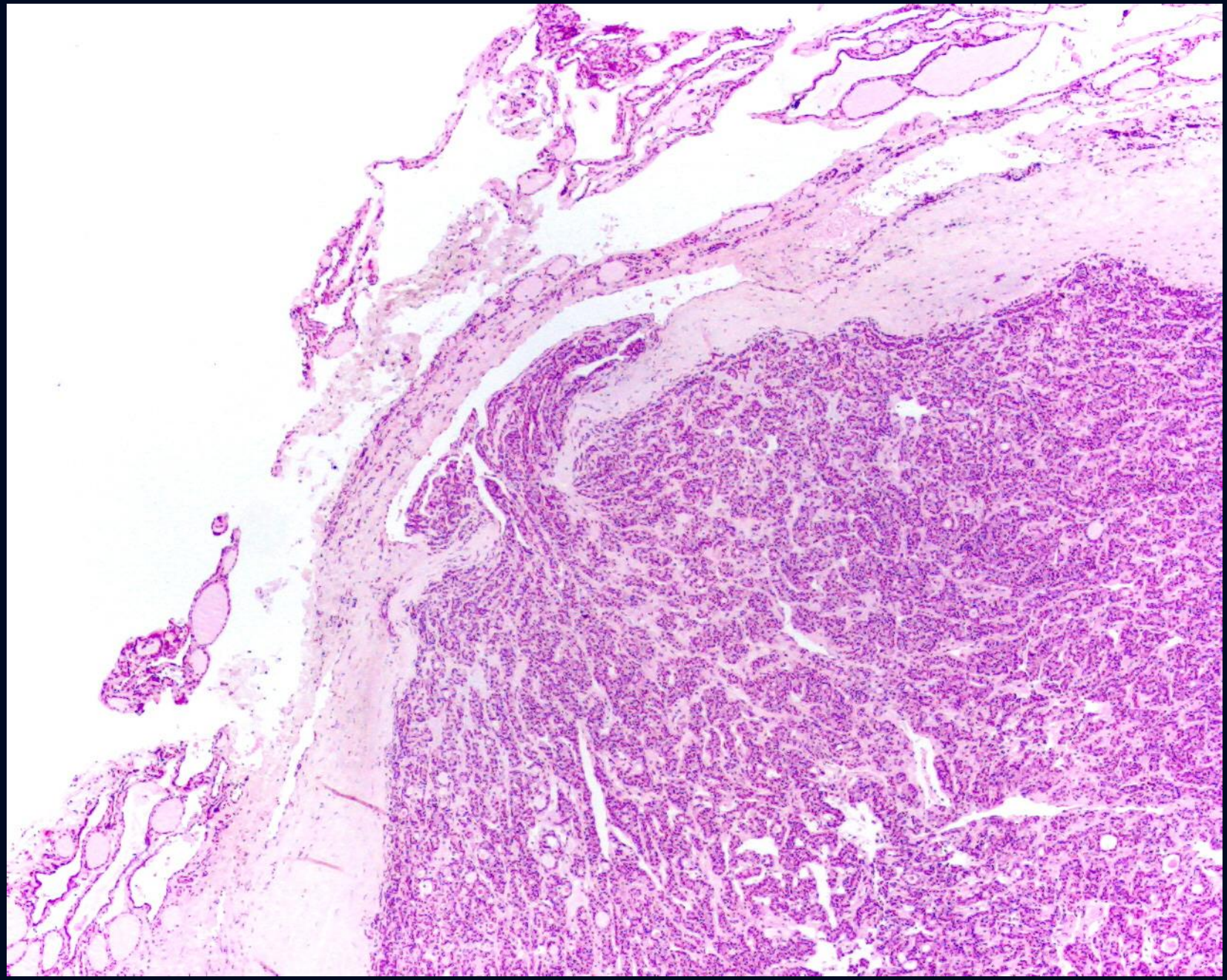
Nuclear features

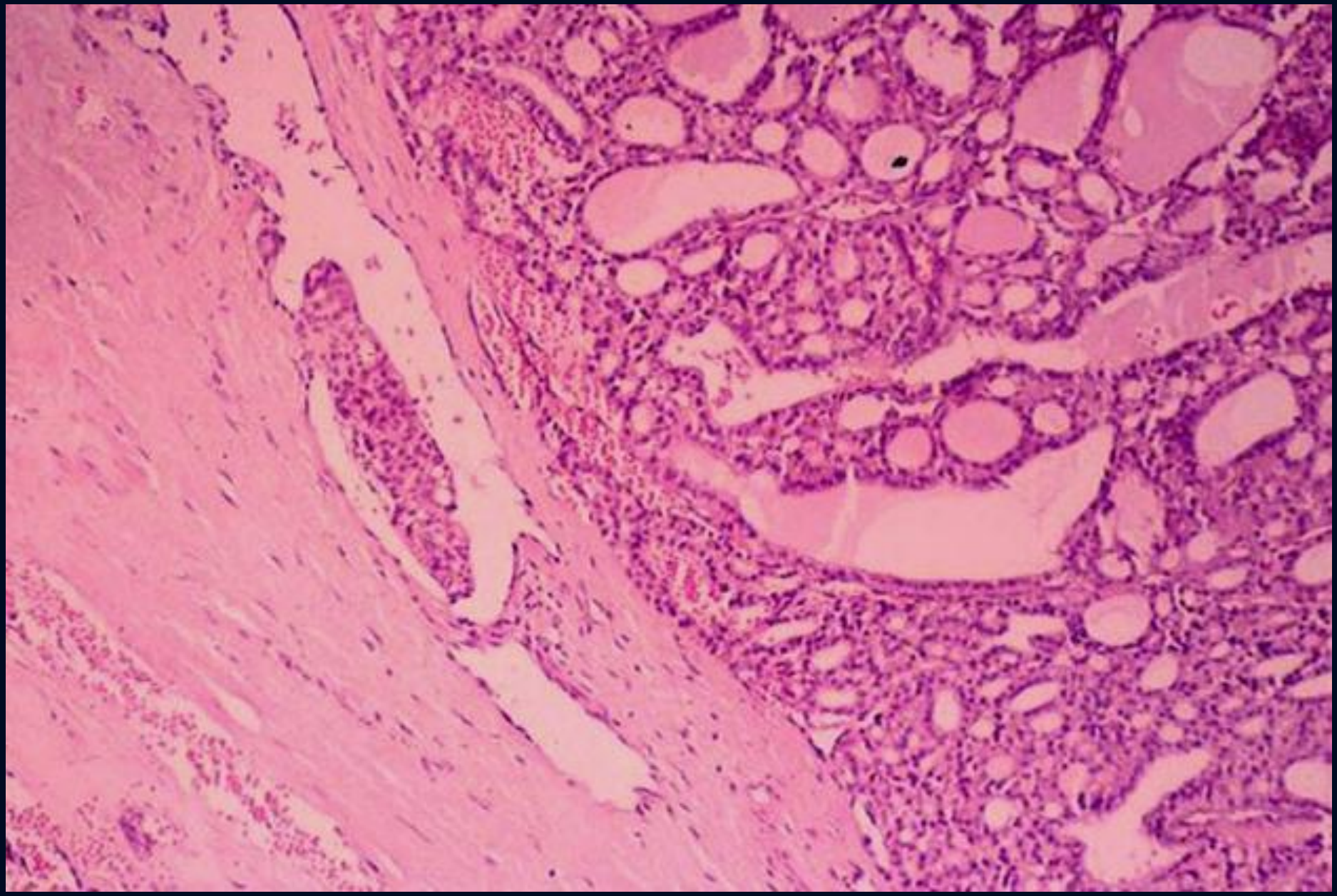


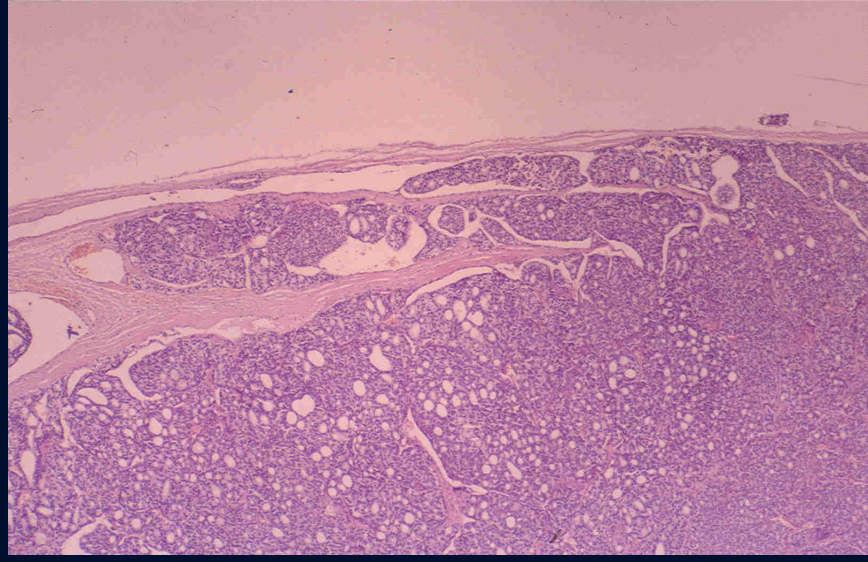
Malignancy in follicular patterned thyroid tumours (follicular variant of papillary carcinoma and follicular carcinoma

Capsular and/or VASCULAR INVASION

Pattern of growth	Solid, insular, trabecular Embryonal, fetal Normofollicular Macrofollicular
Nuclear features	PTC NUCLEI







What is the best way to diagnose parenchymatous/vascular invasion?

Cytopathology	No
Histopathology	Yes
Detection of biomarkers in the plasma/blood	May be
Conventional molecular pathology and high throughput approaches	No

DIAGNOSTIC HINTS

Capsular or, more importantly, vascular invasion
Nuclear features

WHAT ABOUT QUESTIONABLE CASES?

DOES IMMUNOHISTOCHEMISTRY OR MOLECULAR BIOLOGY HELP? **NO**

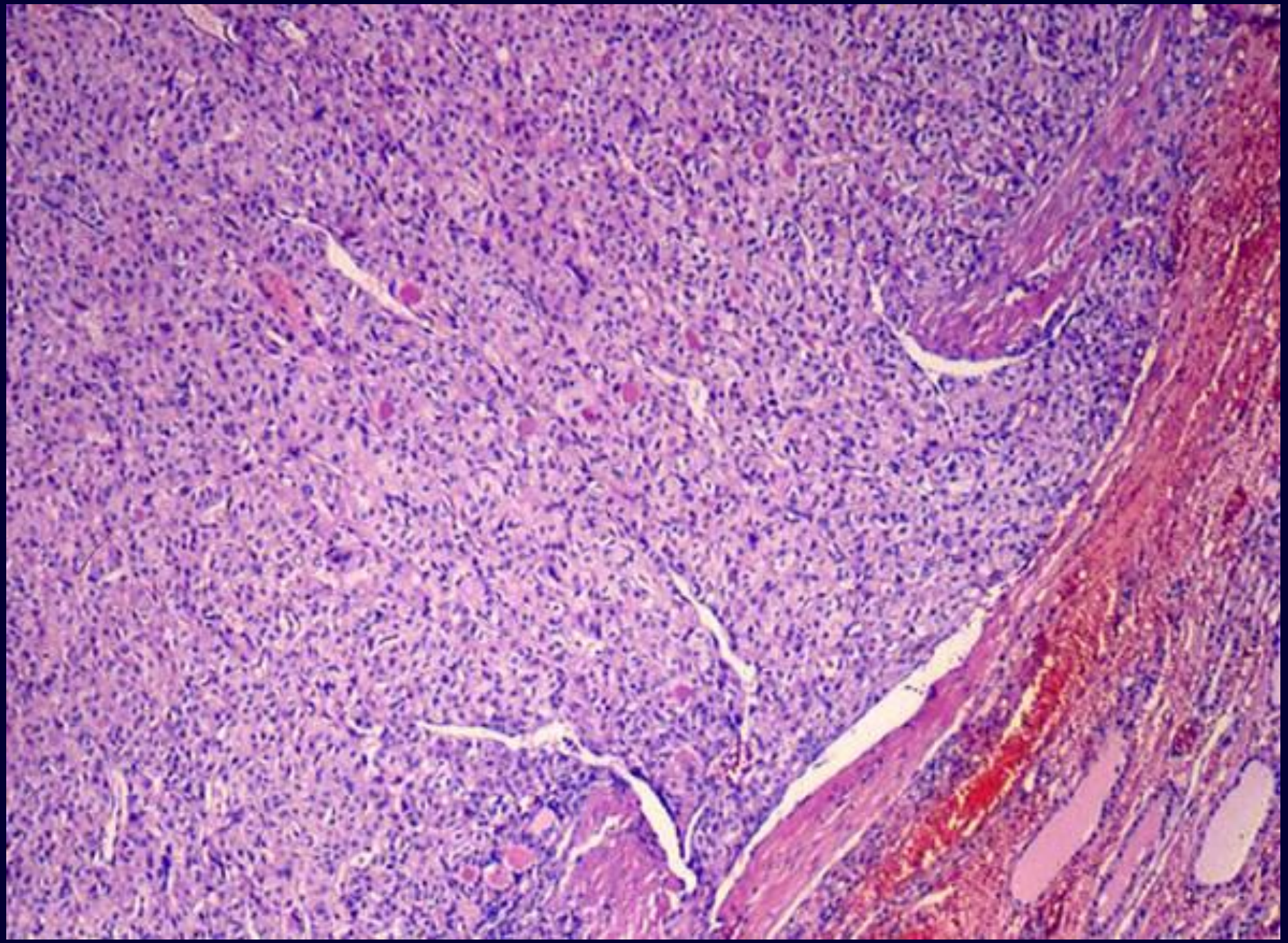
What about high throughput results?

Expression profiling of human tumours: The end of surgical pathology?

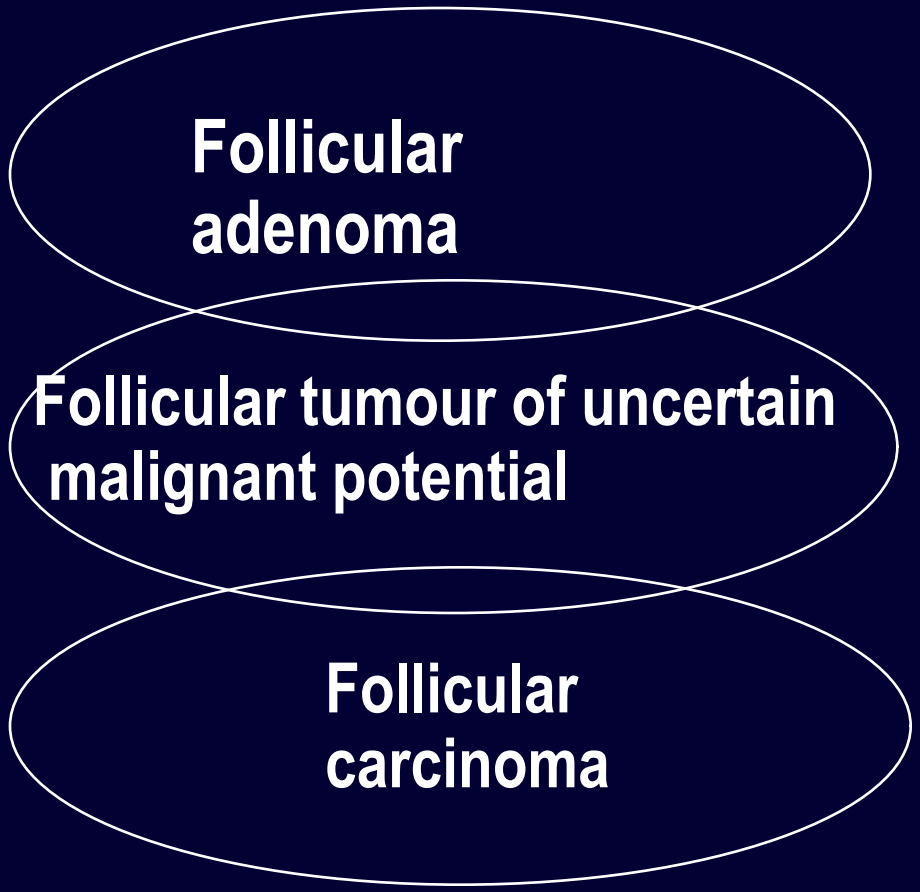
Kadanyi et al, J Mol Diagn 3:92, 2001

The answer is No: Microarrays, genome-wide, miRNAs,… did not provide so far anything really positive

WHAT ABOUT FOLLICULAR-CELL TUMOURS WITH EQUIVOCAL CAPSULAR INVASION?

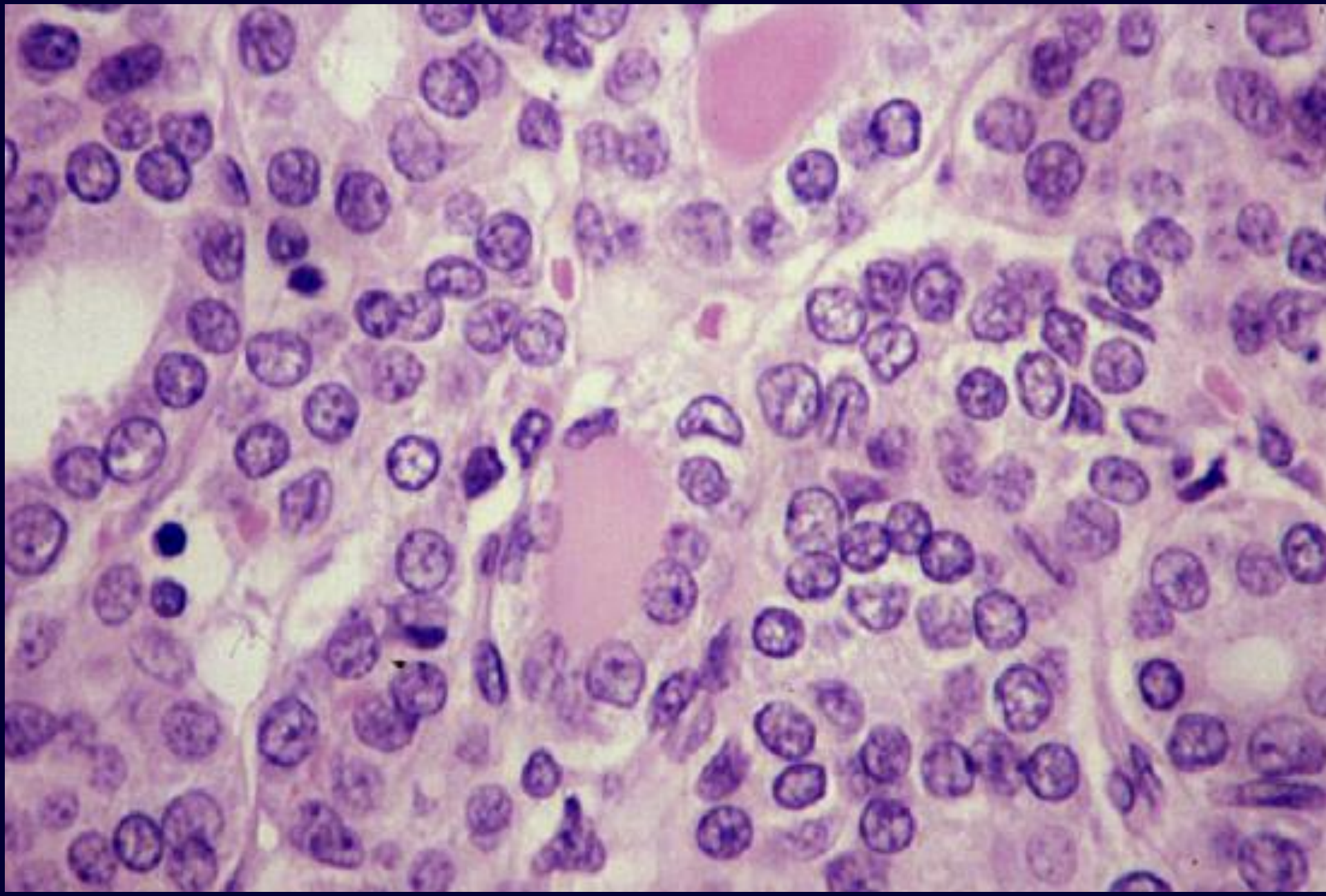


Benign vs Malignant



Williams et al, Int J Surg Pathol, 8:181, 2000
WHO book on Endocrine Tumours, 3rd ed, 2004

WHAT ABOUT FOLLICULAR TUMOURS WITH INTERMEDIATE NUCLEI?



- Well differentiated tumour of uncertain malignant potential

- Well differentiated carcinoma, NOS (If there is invasion)

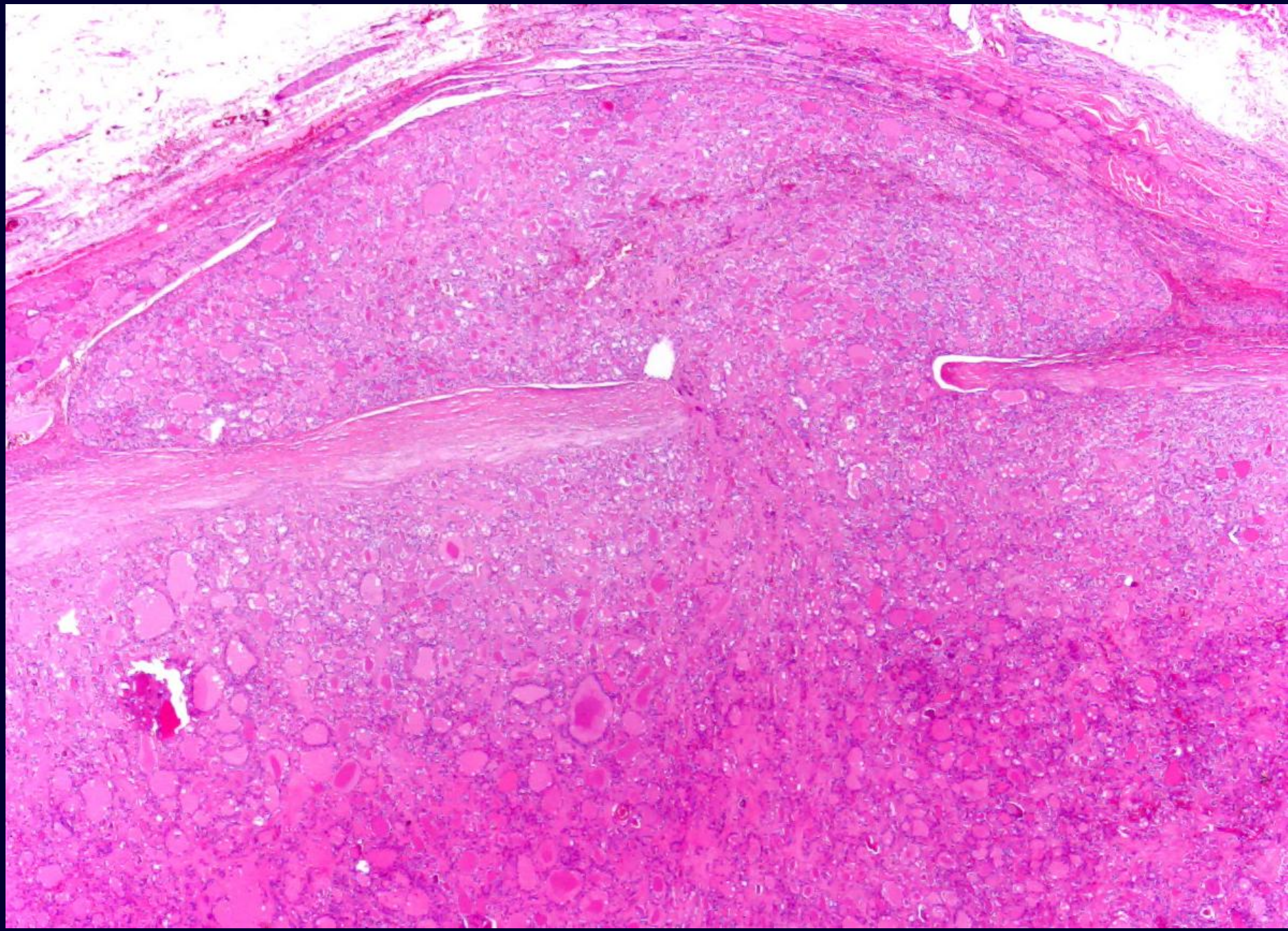
Williams et al, Int J Surg Pathol 8:181, 2000
WHO book on Endocrine Tumours, 3rd edition, 2004

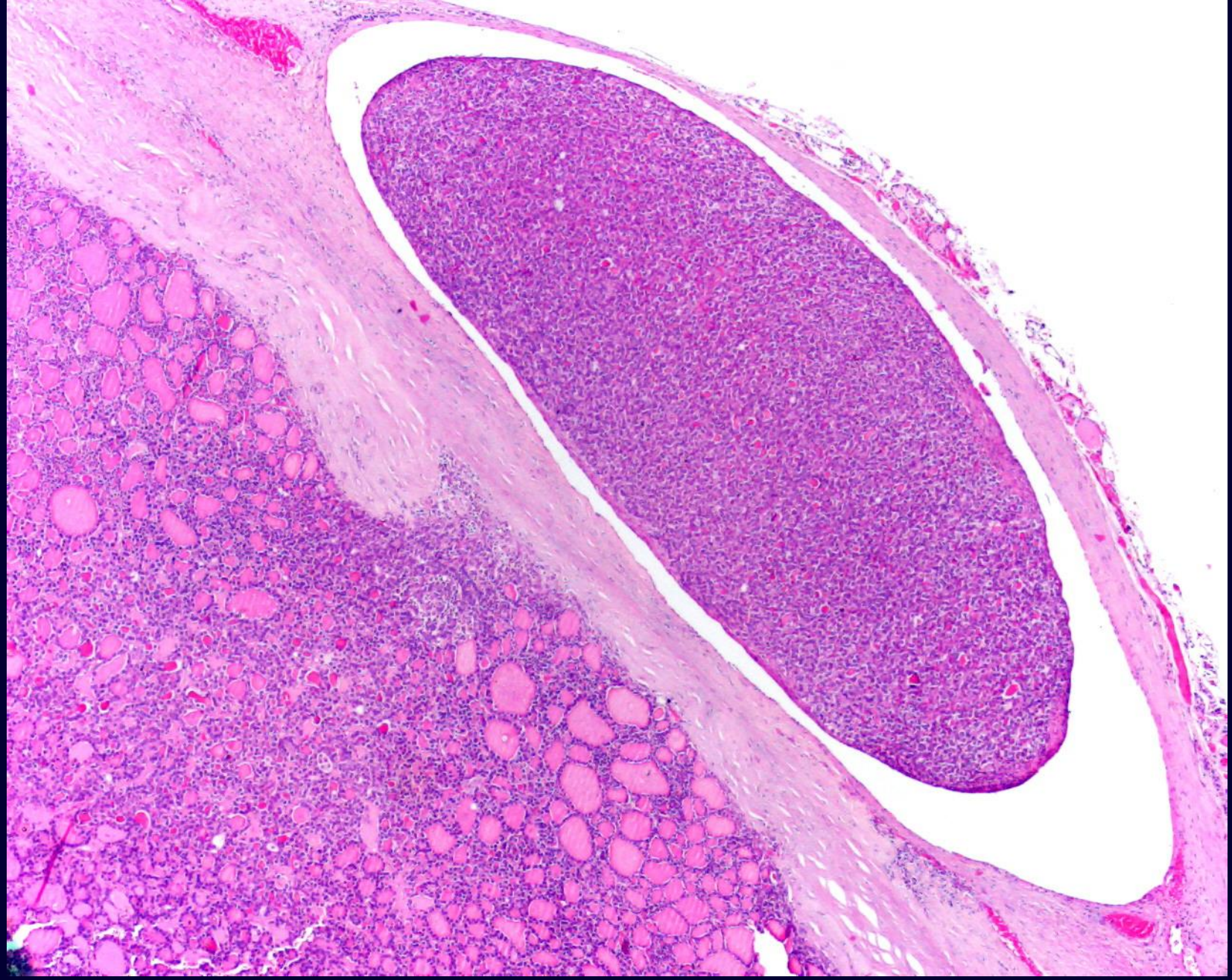
Malignancy in Hürthle cell tumours

Diagnostic hints

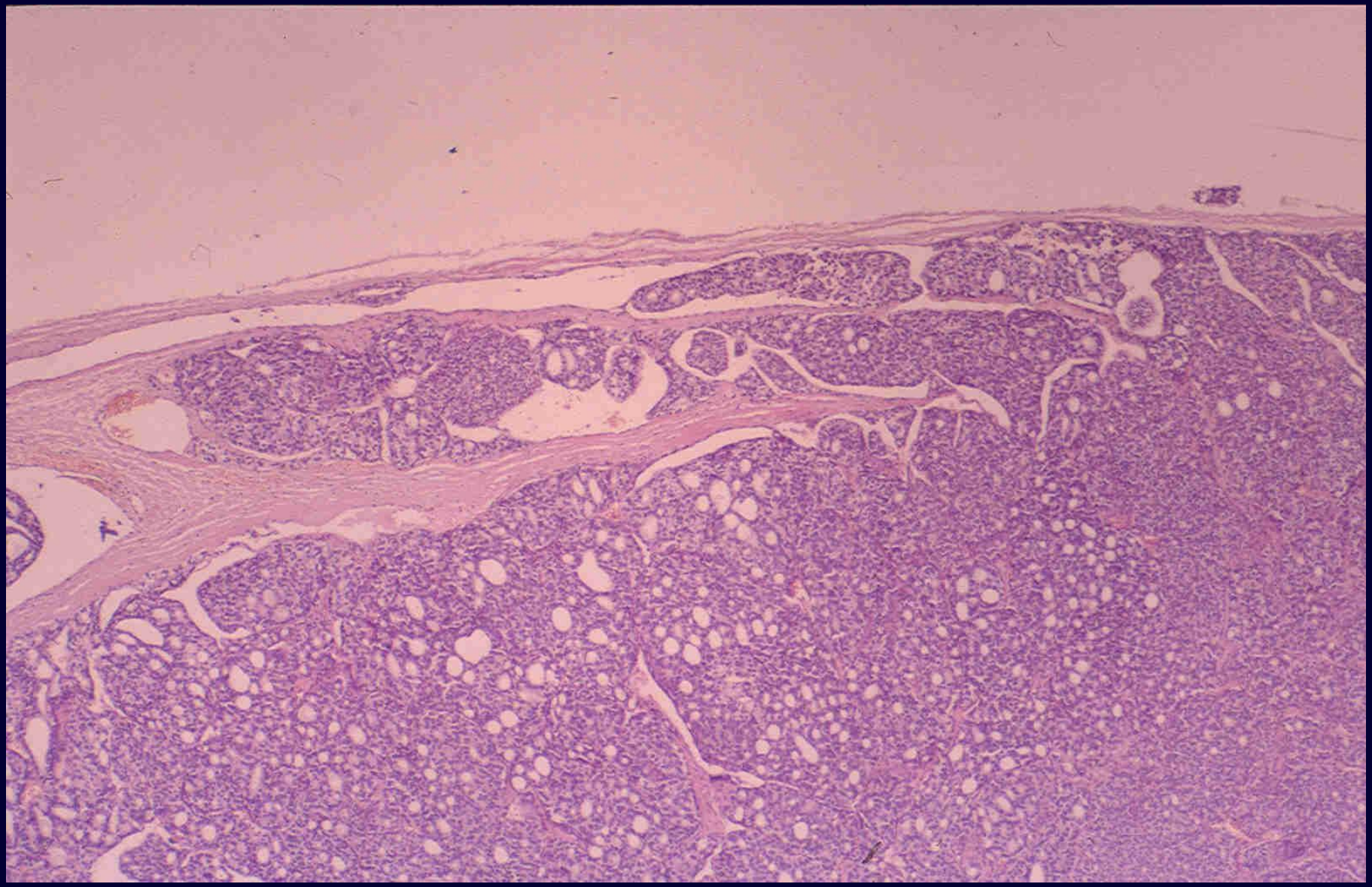
Capsular/vascular invasion

Nuclear features





Follicular carcinoma



Follicular carcinoma

Rosai et al, 2004
WHO book on Endocrine Tumours, 2004

Major problems in thyroid oncology

- Separation of follicular cell from C-cell derived tumours
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PROGNOSTIC FACTORS IN PAPILLARY AND FOLLICULAR THYROID CARCINOMA

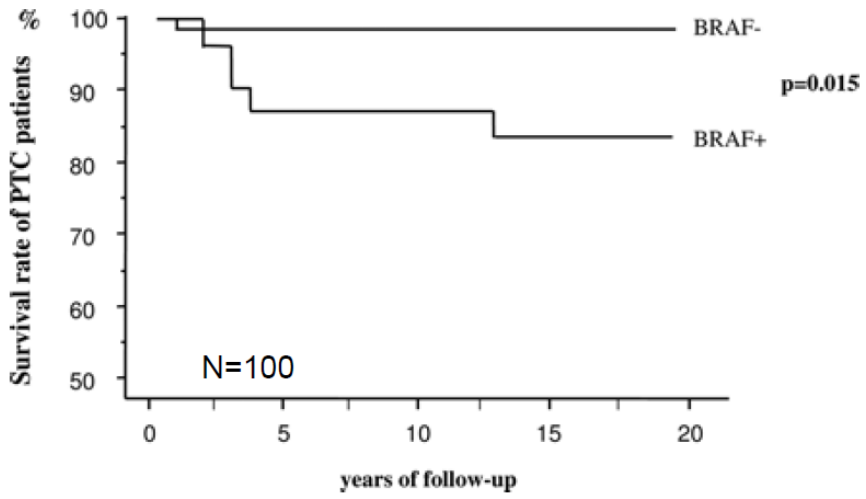
Completeness of surgery and responsiveness to radioactive iodine

A – Age
M – Distant metastases
E – Extrathyroid extension
S – Size of the tumours

Vascular invasion

Still debatable: aneuploidy (D…AMES) and molecular features (MIB1, p53, BRAF)

BRAF Predicts Tumor-Related Mortality



Elisei R et al. JCEM (2008)

Most studies did not confirm the worse prognosis of BRAF mutated tumours provided major clinico-pathological features are controlled

Virchows Arch
DOI 10.1007/s00428-011-1133-7

ORIGINAL ARTICLE

The preeminence of growth pattern and invasiveness and the limited influence of *BRAF* and *RAS* mutations in the occurrence of papillary thyroid carcinoma lymph node metastases

Catarina Eloy · Joana Santos · Paula Soares · Manuel Sobrinho-Simões

pT Extrathyroid extension

Infiltrative growth pattern

Vascular invasion

Multicentricity

BRAF V600E mutation did not play a significant role

Eloy et al, Virchows Arch, 2011 (in press)

Few studies have reported the BRAF status of well differentiated, distantly metastatic papillary thyroid carcinomas

Liu RT et al. Clin Endocrinol 63:461, 2005

Fugazzola R et al. Endocr Relat Cancer 13:455, 2006

Costa AM et al. Clin Endocrinol 68:618, 2008

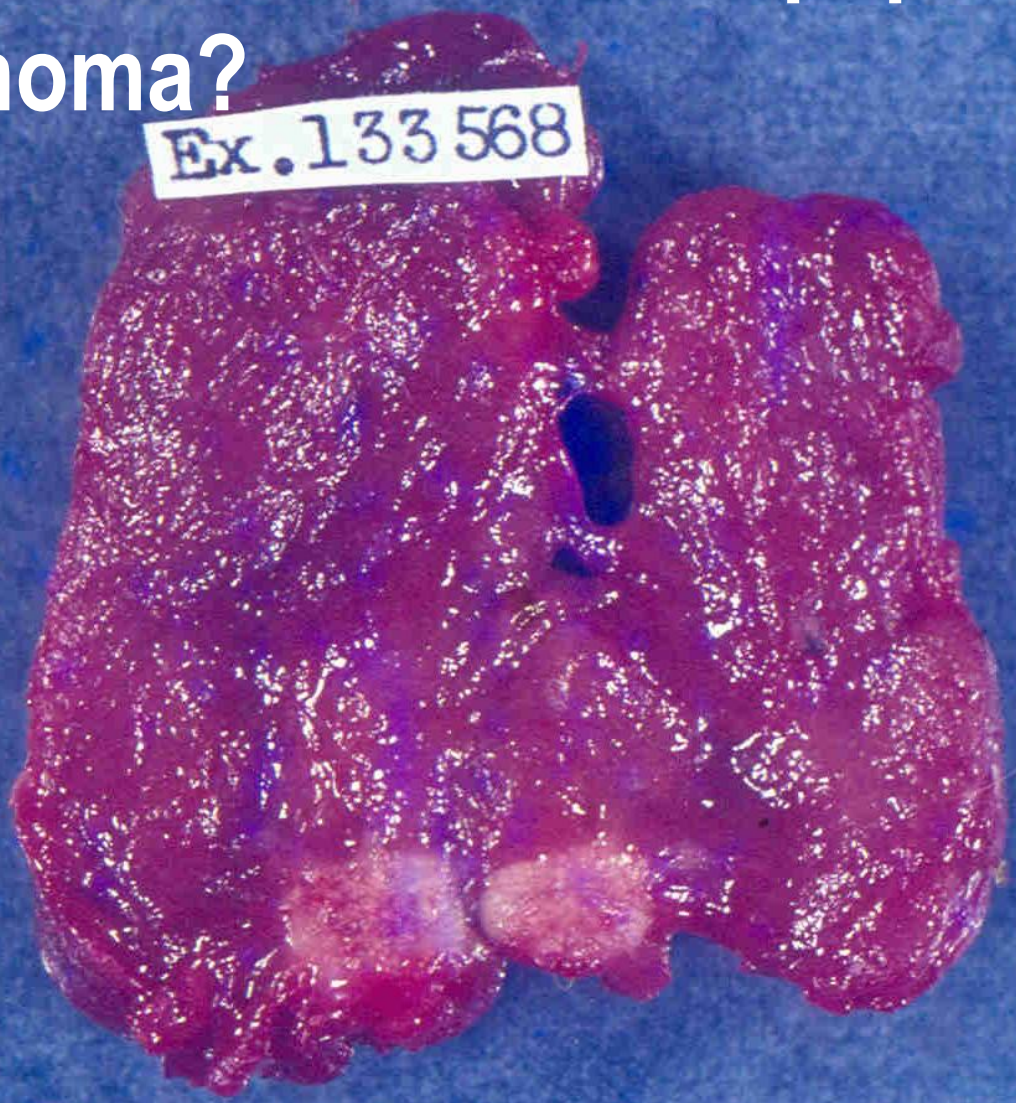
Eloy C et al. Virchows Arch 459:256, 2011

Only 4 out 20 (20%) PTCs had the BRAF V600E mutation

Conclusion:

BRAF V600E mutation should not be considereed, *per se*, a negative prognostic marker in PTC.

What about BRAF mutation in papillary microcarcinoma?



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BRAF and Aggressiveness of Papillary MicroCA

Table 1 Analysis of $BRAF^{V600E}$ mutation in PTMCs

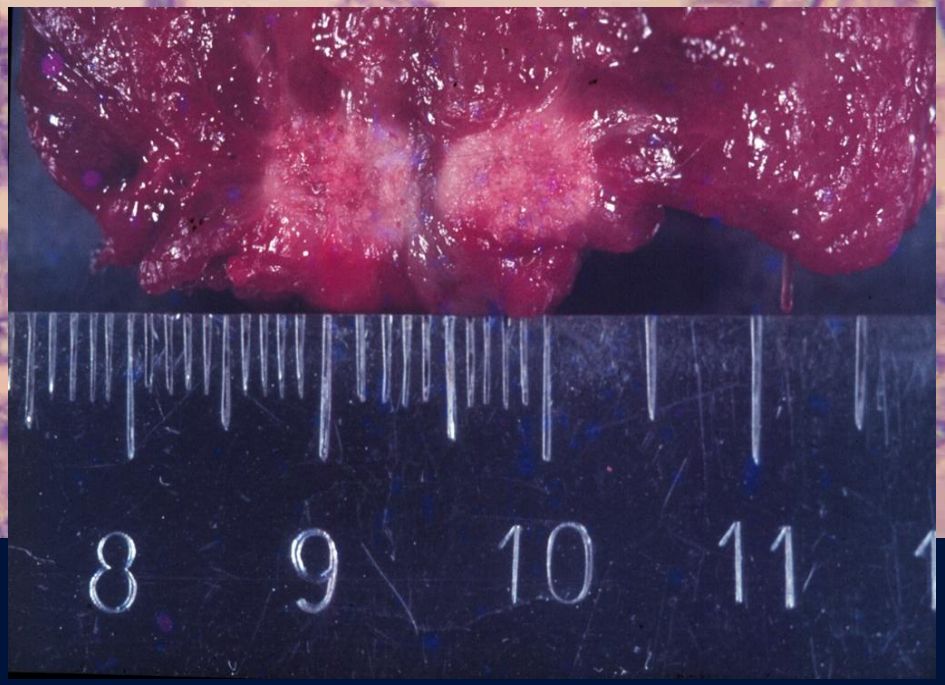
	BRAF mutation		*p*
	Positive (*n* = 24)	Negative (*n* = 40)	
Age (yr)	49.1 ± 10.4	45.1 ± 11.2	0.533
Male:female ratio	2:22	6:34	0.358
Nodular goiter	6 (25%)	8 (20%)	0.432
Hashimoto thyroiditis	0	8 (20%)	>0.99
Extrathyroidal extension	12 (50%)	4 (10%)	0.001*
Nodes metastasis	12 (50%)	6 (15%)	0.003*
Tall cell variant	2 (8.3%)	2 (5%)	0.483
T_1:$T_{3/4}$ ratio	12:12	36:4	0.001*

* Statistically significant

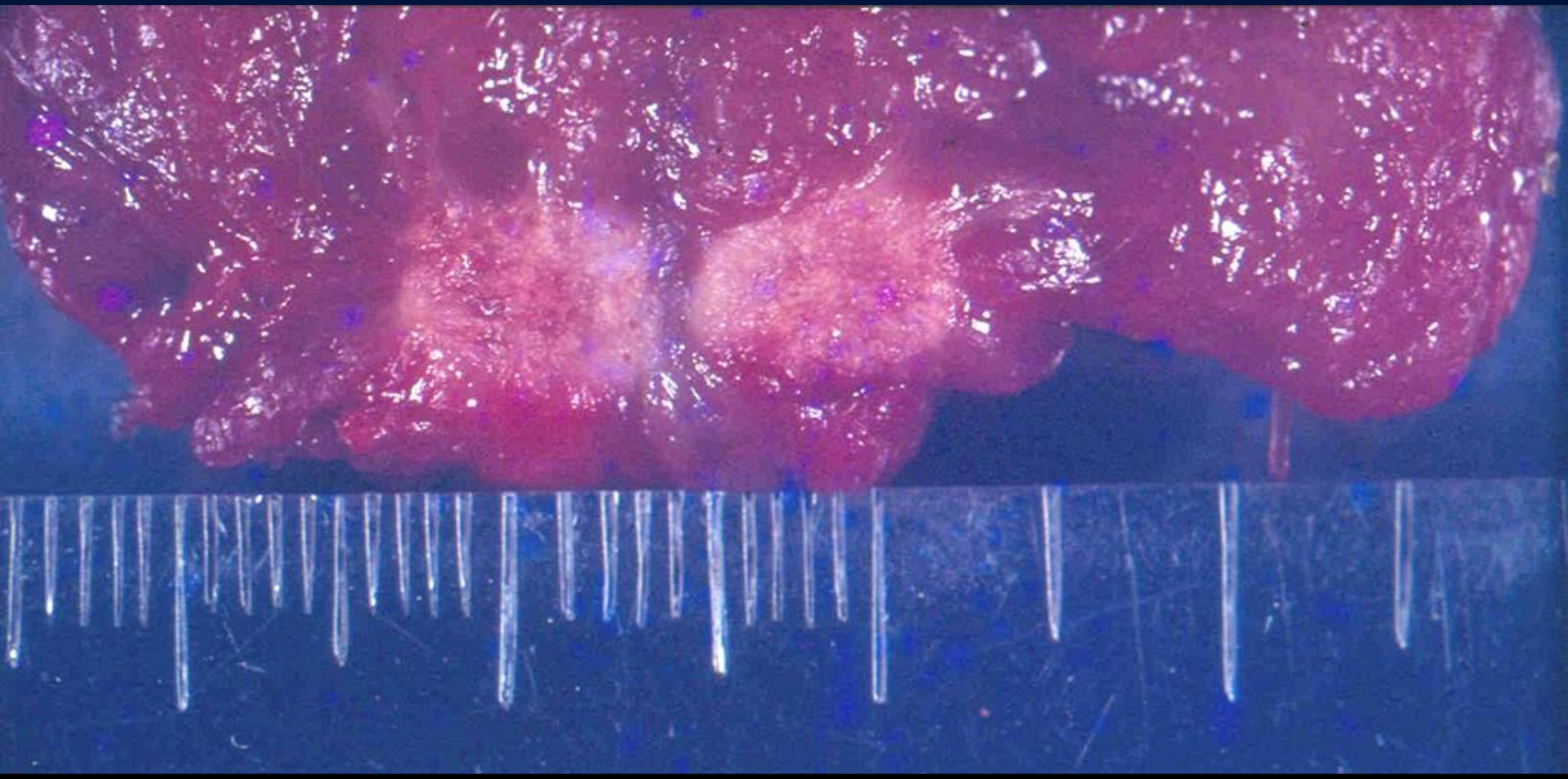
Lee X et al. *Ann Surg Oncol* (2009)

BRAF mutation in about 20-40% of papillary microcarcinomas
Should they be treated aggressively?

Soares and Sobrinho-Simões, Future Oncol, 2009



Size, type of growth and invasiveness features



There is no solid evidence to support the utilization of the 10mm size as a dividing line between extremely low and low risk papillary thyroid microcarcinomas **(We guess the line might have been drawn at 6 or 12mm in case we had 6 fingers in each hand).**

Soares and Sobrinho Simões, Nature Rev Endocrinol, 2011

CANCER

Small papillary thyroid cancers—is *BRAF* of prognostic value?

Paula Soares and Manuel Sobrinho-Simões

We think the excellent prognosis of microPTC means that it is unrealistic to suggest, as it has been recently advanced,[10] that patients with *BRAF* mutated microPTC should be treated more aggressively just taking into consideration the BRAF status.

Nature Reviews Endocrinology, 2011

Original Article

A Combined Molecular-Pathologic Score Improves Risk Stratification of Thyroid Papillary Microcarcinoma

Leo A. Niemeier, MD[1]; Haruko Kuffner Akatsu, MD[2]; Chi Song, MS[3]; Sally E. Carty, MD[4]; Steven P. Hodak, MD[2]; Linwah Yip, MD[4]; Robert L. Ferris, MD, PhD[5]; George C. Tseng, PhD[3]; Raja R. Seethala, MD[1]; Shane O. LeBeau, MD[2]; Michael T. Stang, MD[4]; Christopher Coyne, MD[2]; Jonas T. Johnson, MD[5]; Andrew F. Stewart, MD[2]; and Yuri E. Nikiforov, MD, PhD[1]

3 histopathologic features: superficial tumor location, intraglandular tumor spread/multifocality, and tumor fibrosis cooperate with BRAF mutations.

DOI: 10.1002/cncr.26425, **Received:** May 4, 2011; **Revised:** June 16, 2011; **Accepted:** June 20, 2011, **Published online** in Wiley Online Library (wileyonlinelibrary.com)

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Still debatable: aneuploidy (D…AMES) and molecular features (MIB1, p53, BRAF)

- Benign *vs* Malignant *vs* Borderline (Uncertain malignant potential – UMP)
- Lobectomy *vs* Lobectomy plus isthmectomy *vs* Total thyroidectomy
- Radioactive iodine: Yes or No?
- Targeted therapies: When and Which one(s)?

Cytology
Histology
+
Molecular Pathology

The BRAF^{V600E} Oncogene Induces Transforming Growth Factor β Secretion Leading to Sodium Iodide Symporter Repression and Increased Malignancy in Thyroid Cancer

Garcilaso Riesco-Eizaguirre,[1,2] Irene Rodríguez,[1] Antonio De la Vieja,[1,4] Eugenia Costamagna,[1] Nancy Carrasco,[5] Manuel Nistal,[3] and Pilar Santisteban[1]

Cancer Res 2009; 69: (21). November 1, 2009

mTOR pathway overactivation in BRAF mutated papillary thyroid carcinoma

Faustino A,…Soares P. J Clin Endocrinol Metabol, 2012 (in press)

"mTOR overactivation induces repression of Sodium Iodide Symporter (NIS)"

Revision of 401 cases of primary papillary and follicular carcinomas displaying increased clinical aggressiveness and not responding to radioactive iodine therapy

2/5 Oncocytic (Hürthle cell) variant of papillary carcinoma

1/5 Oncocytic (Hürthle cell) variant of follicular carcinoma

1/5 Poorly differentiated carcinoma

1/5 Other histotypes

Sobrinho-Simões M & Feldman M, Unpublished observations, 2012

Study of genetic alterations on follicular Hürthle cell tumours

De Vries MM et al. Histopathology, 2012 (in press)

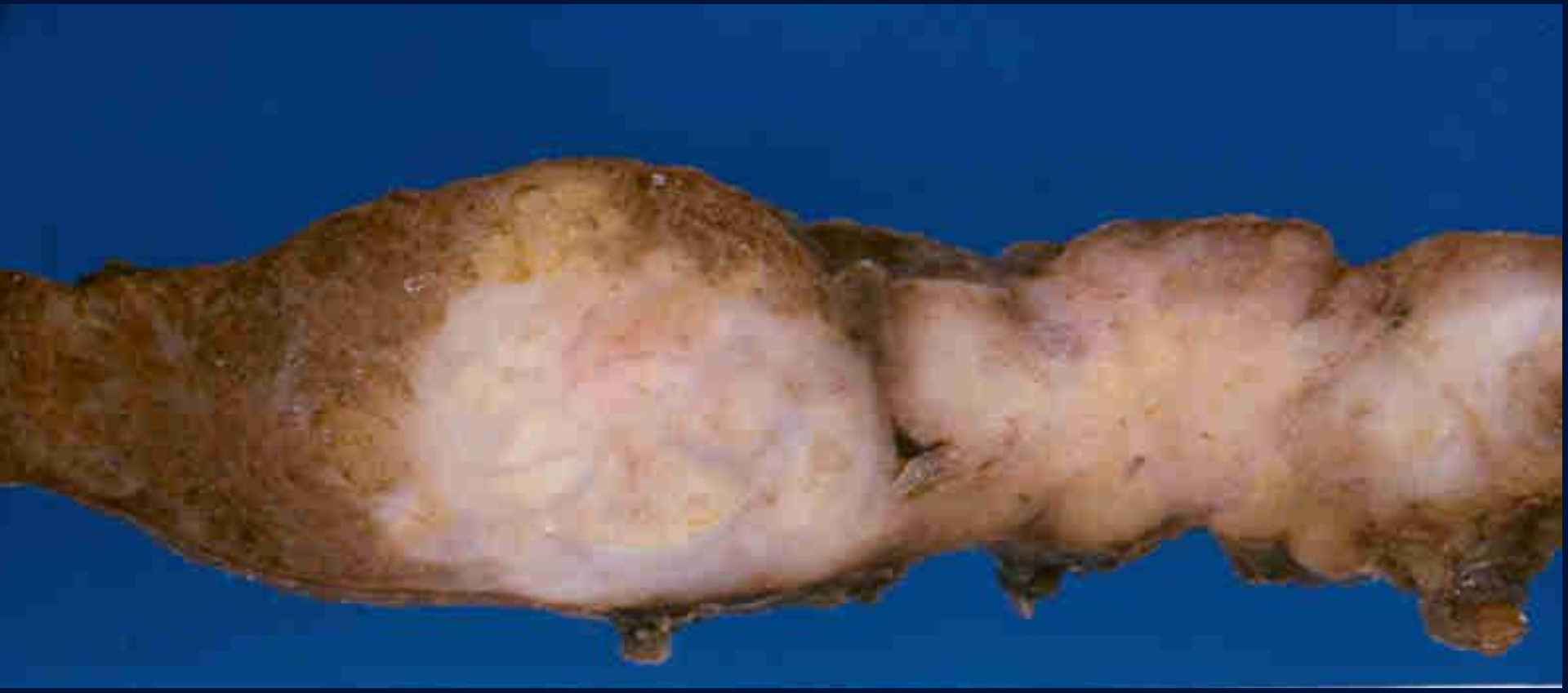
The biology and the genetics of Hürthle cell tumors of the thyroid

Maximo V et al. Endocrine-Related Cancer, 2012 (in press)

“RET/PTC rearragements are frequently detected in every type of Hürthle cell tumour“

Therapeutic problems

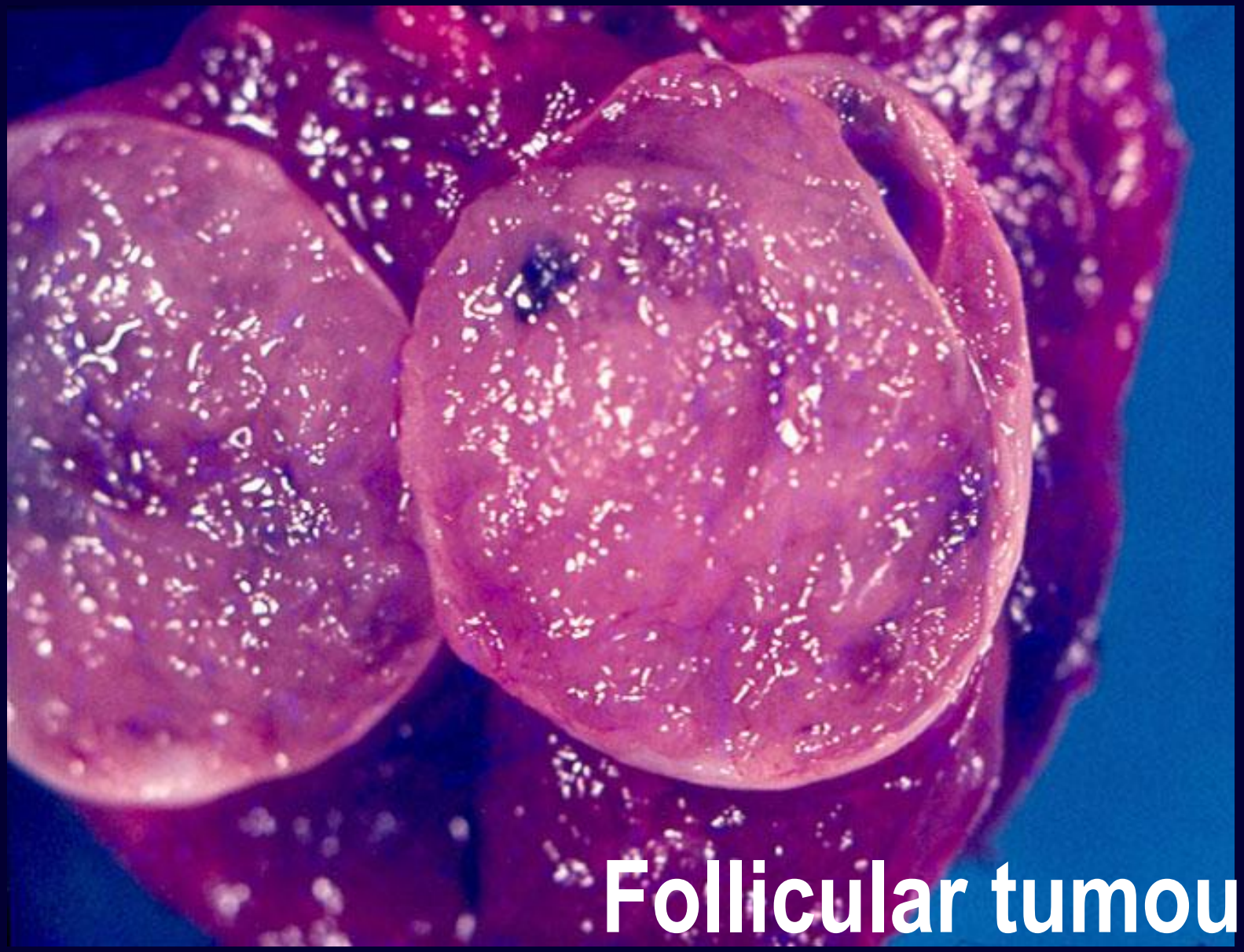
Phenotypic and genotypic heterogeneity and topography



It will be necessary to target the ERK/MAPK (RET, BRAF, RAS,…) and/or the PI3K/AKT/mTOR and/or JAK/STAT3 pathways, as well as mitochondrial and metabolic alterations

SÃO JOÃO
HOSPITAL

20 IPATIMUP
Instituto de Patologia e Imunologia Molecular da Universidade do Porto

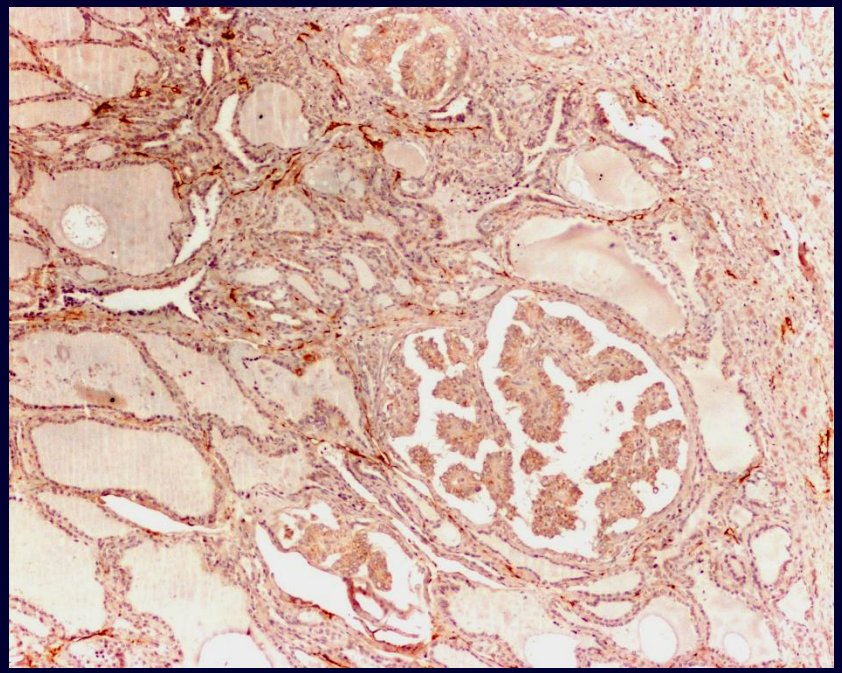

Follicular tumours

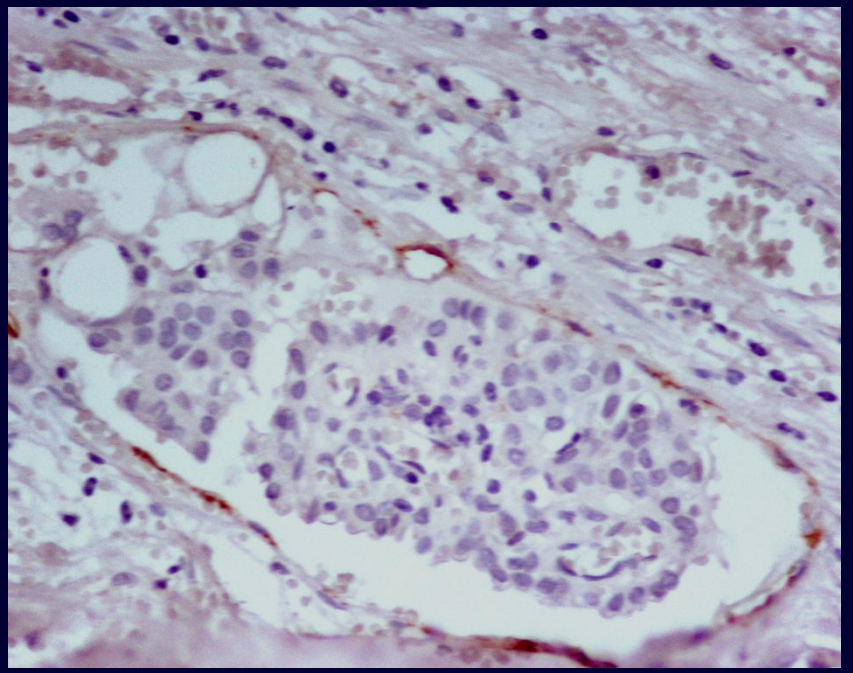
Topography and neoplastic tissue features

Intratumoural lymph vessel density is related to presence of lymph node metastases and separates encapsulated from infiltrative papillary thyroid carcinoma

Eloy C [1,2,3,4], Santos J[3], Soares P [2,3], Sobrinho-Simões M [1,2,3].

Virchows Archiv, in press





Intratumor heterogeneity and branched evolution revealed by multiregion sequencing

Gerlinger M,… Swanton C. N Engl J Med 366:883, 2012

"Multiple spatially separated samples obtained from primary renal carcinomas and associated metastatic sites"

"63 to 69% of all somatic mutations" (oncogenes, tumor-supressor genes and landscaper genes) "not detectable across every tumor region"

"Gene-expression signatures of good and poor prognosis were detected in different regions of the same tumor"

BRAF V600E mutation does not mean distant metastasis in thyroid papillary carcinomas (PTC)

Sancisi V et al. J Clin Endocrinol Metab (in press)

- BRAF V600E mutation was present in 29.8% of 47 well differentiated, distantly metastastic, PTCs and in 44.0% of 75 non-agressive control PTCs

BRAF mutation associated with other genetic events identifies a subset of aggressive papillary thyroid carcinoma.

Costa AM et al. Clin Endocrinol (Oxf) 68:618, 2008

Few studies have reported the BRAF status of distantly metastatic, well differentiated, papillary thyroid carcinomas

Liu RT et al (2005); Fugazzola R et al (2006); Costa AM et al (2008); Eloy C et al (2011)

Only 4 out 20 (20%) PTCs (had the BRAF V600E mutation

Oncocytic (Hürthle) follicular-cell derived tumours (adenoma, follicular carcinoma and oncocytic variant of PTC) revealed separated clusters of miRNA expression profiles thus supporting the existence of a fairly specific oncocytic (Hürthle) cell phenotype.

Nikiforova et al, JCEM 93:1600, 2008 & Endocr Pathol 20:851, 2009

Intratumoural lymph vessel density is related to presence of lymph node metastases and separates encapsulated from infiltrative papillary thyroid carcinoma

Eloy C et al. Virchows Arch 459:595, 2011

- **Intratumoural lymph vessels were almost only detected in papillary or follicular patterned PTCs with an infiltrative pattern of growth.**

- **Intratumoural lymph vessel density (D2-40 expression) correlated with the occurrence of extrathyroid extension, lymph vessel invasion and lymph node metastases in PTCs.**

The preeminence of growth pattern and invasiveness and the limited influence of BRAF and RAS mutations in the occurrence of papillary thyroid carcinoma lymph node metastases.

Eloy C et al. Virchows Arch 459:265, 2011

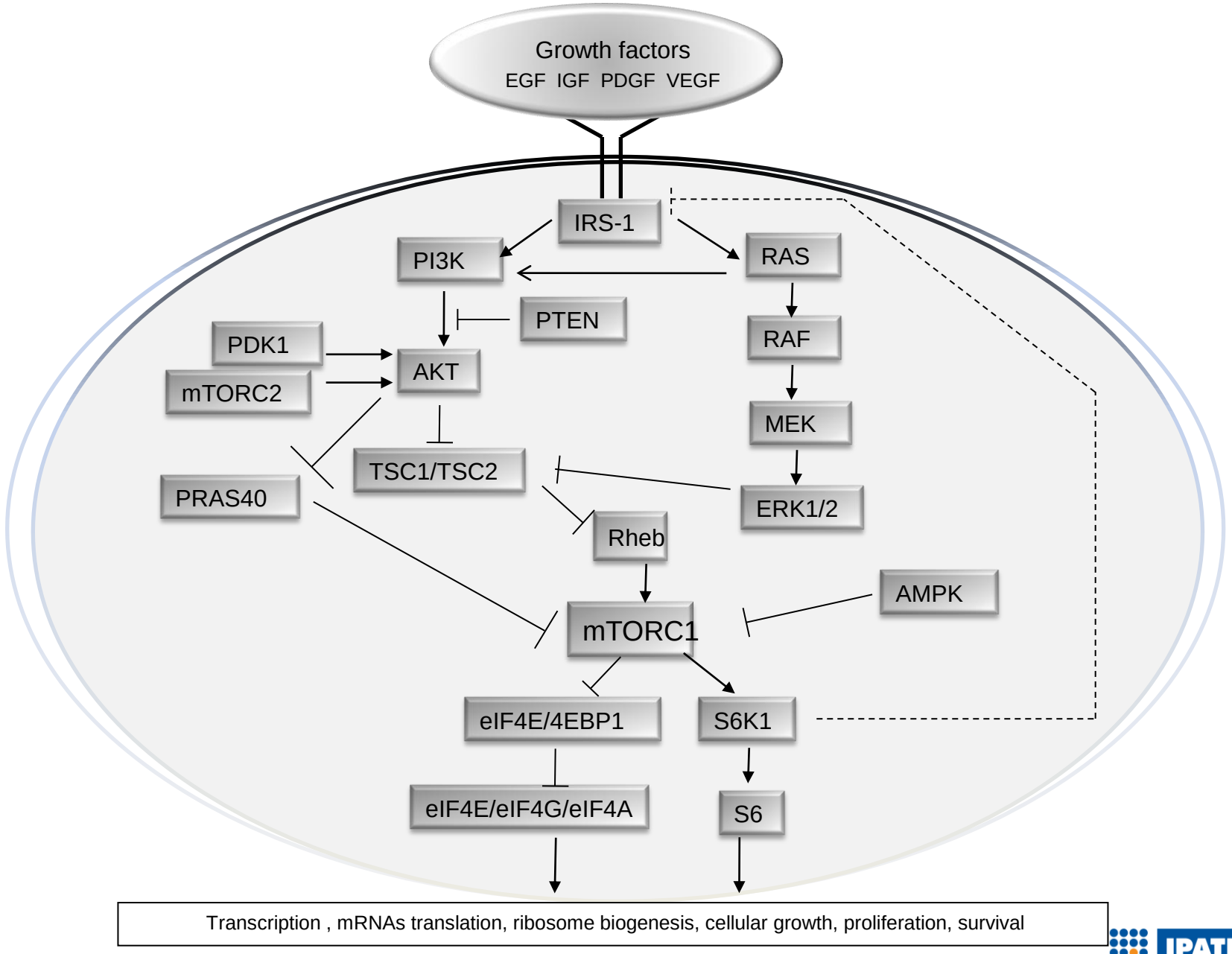
- **The most important prognostic factors were extrathyroid extension and poorly circumscribed growth pattern**

- **BRAF mutation was not significantly associated to nodal metastization**

"STAT3 Negatively Regulates Thyroid Tumorigenesis"

Couto J et al. PNAS, 2012 (in press)

The role of STAT3 was investigated in cell lines and *in vivo* models (xenografts and transgenics) of thyroid cancer. Stable knockdown of STAT3 in TCCs did not alter *in vitro* growth while, *in vivo*, shSTAT3 tumors grew significantly faster than matched controls. The discrepancy observed between *in vivo* and in vitro growth could be due to fact that the presence/absence of STAT3 within tumor cells may alter the levels of paracrine factors leading to the differential recruitment of stromal cells (e.g., mesenchymal cells, including cancer-associated fibroblasts and subtypes of myeloid cells). Similar *in vivo* results were obtained in the murine model of BRAFV600E-induced PTC



THYROID CARCINOMA

Follicular carcinoma
Papillary carcinoma
(Hürthle cell carcinoma)
Medullary carcinoma
Poorly differentiated carcinoma
Undifferentiated carcinoma

WHO books on Endocrine Tumours, 2nd & 3rd editions, 1988 & 2004

Most frequent diagnostic problems of thyroid pathology in a consultancy practice

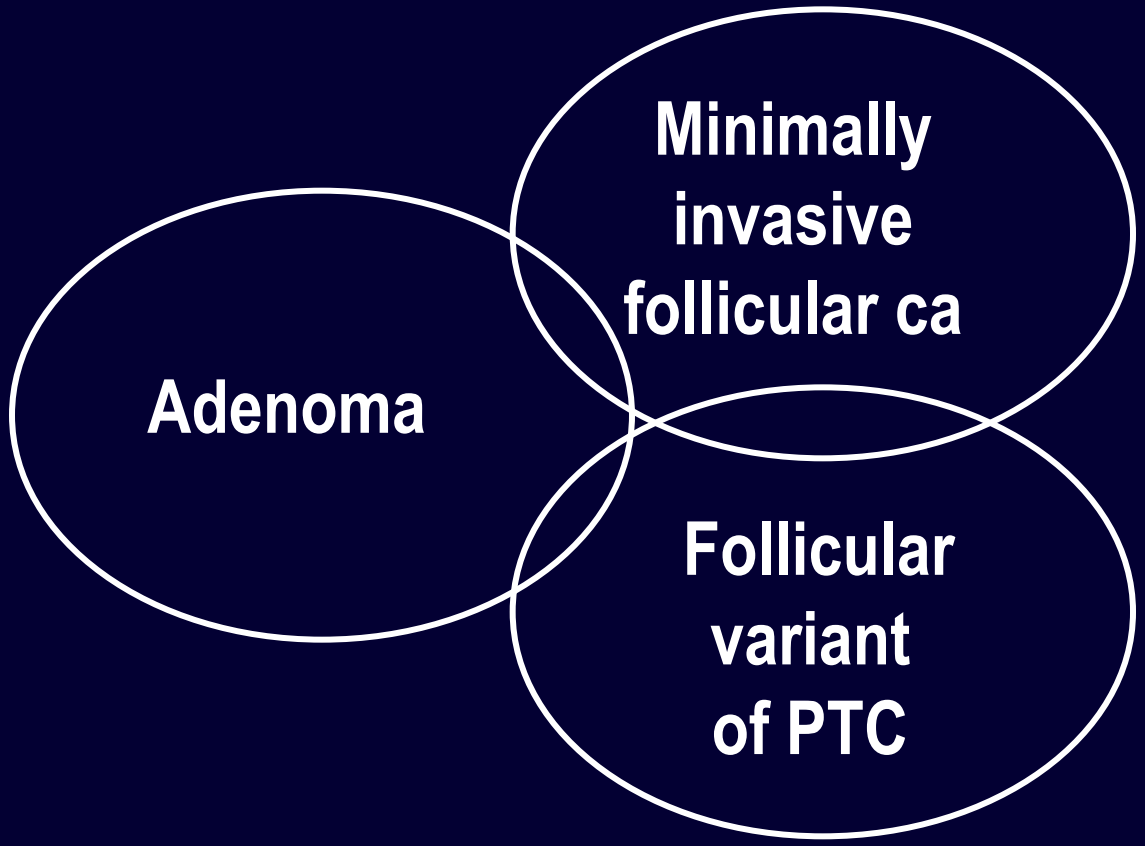
1. Is there a focus (or some foci) of papillary carcinoma in “this” Hashimoto’s thyroiditis or “this” nodular goiter?

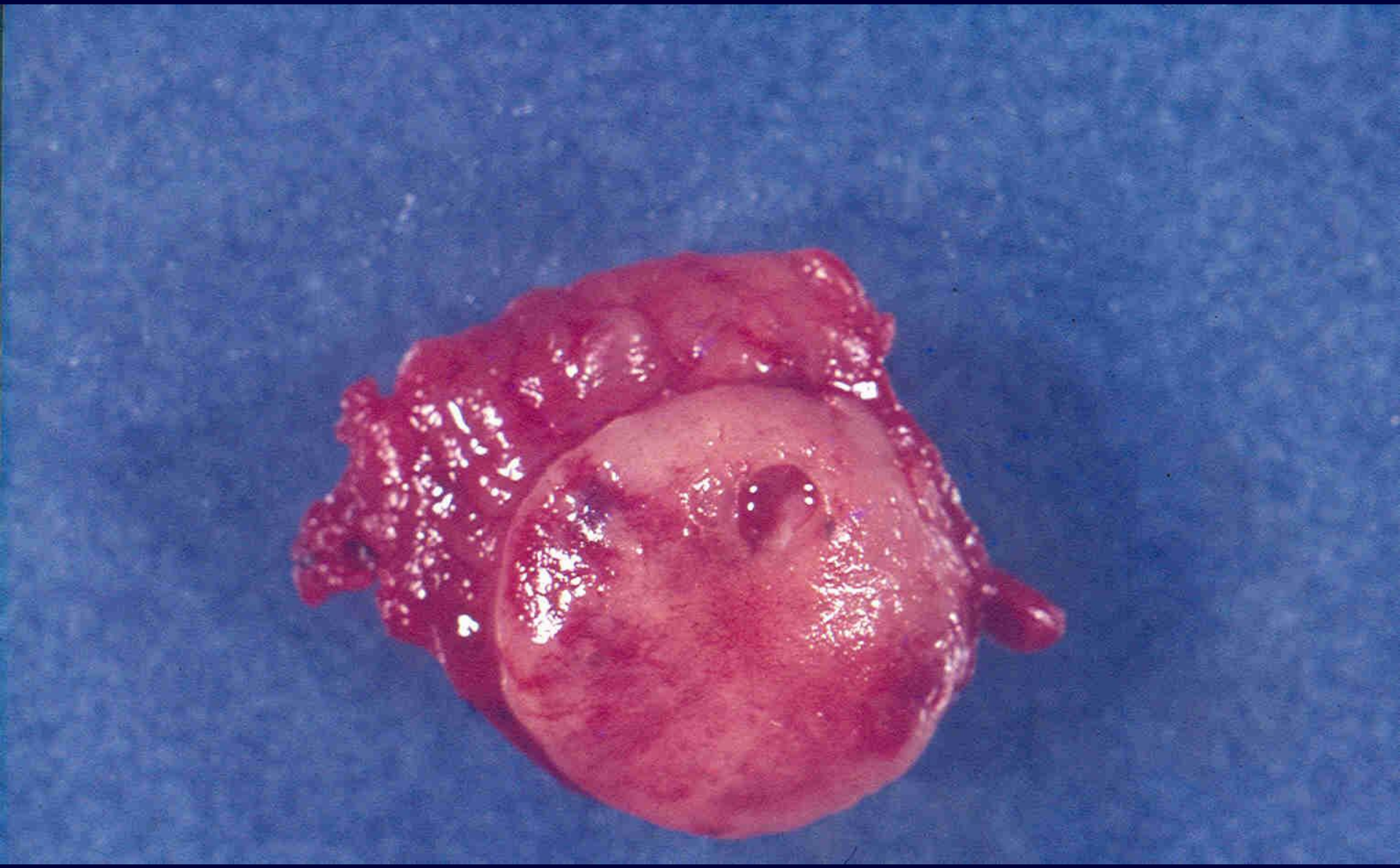
2. Is this lesion an adenoma, a follicular carcinoma or a follicular variant of papillary carcinoma? If you go for follicular carcinoma how would you classify it?

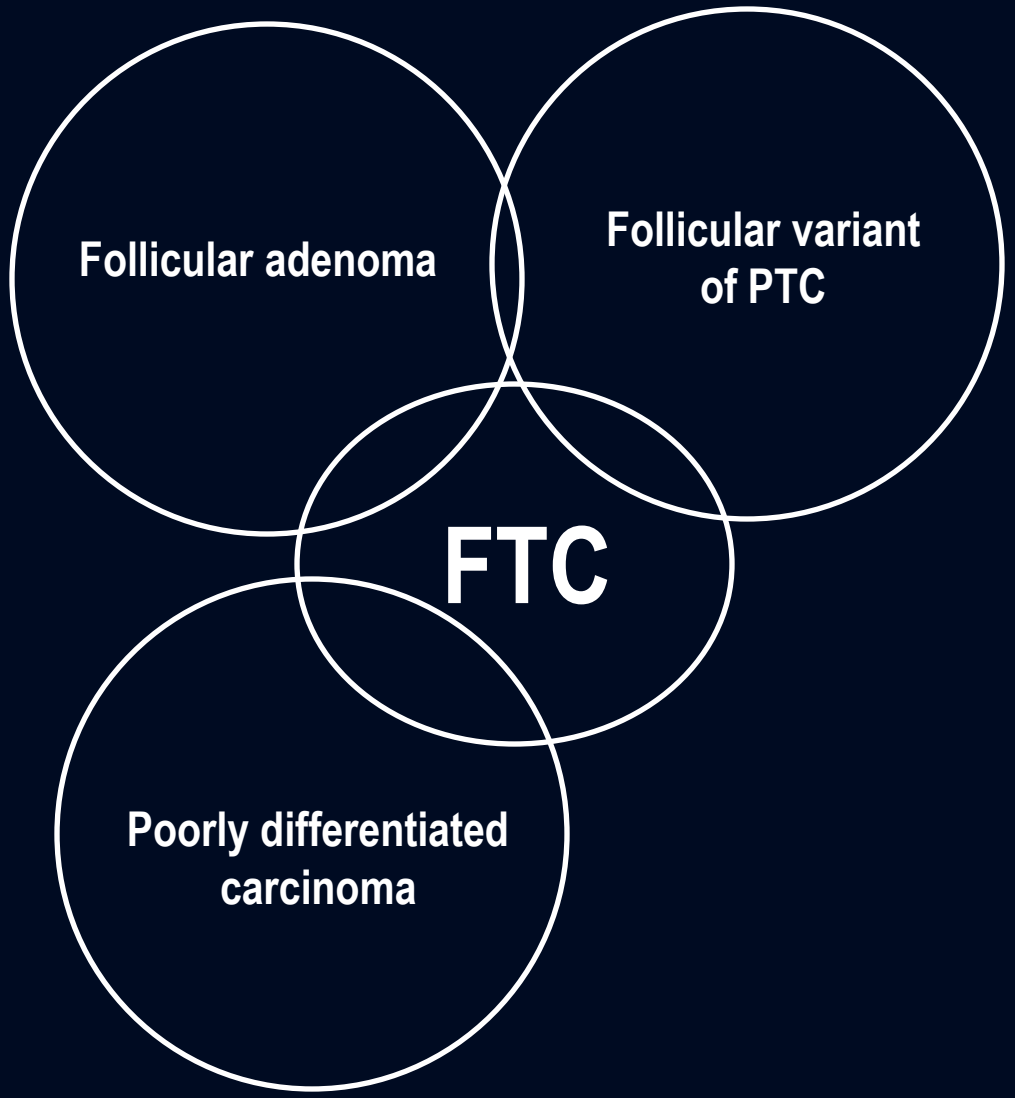
3. Is this a well differentiated carcinoma with a solid pattern of growth or a poorly differentiated carcinoma?

4. How would you classify this Hürthle cell lesion?

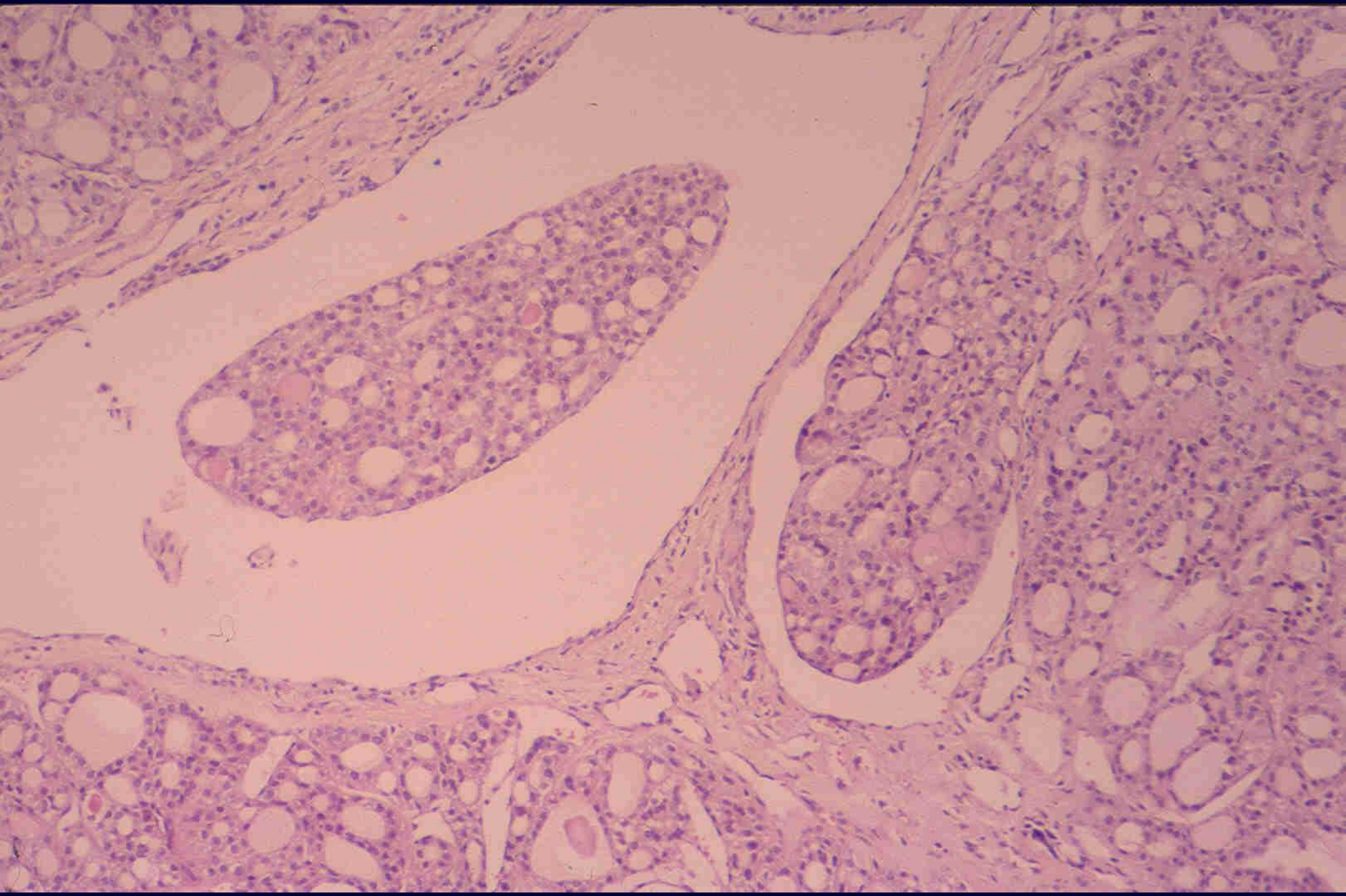
Follicular patterned, encapsulated neoplasms

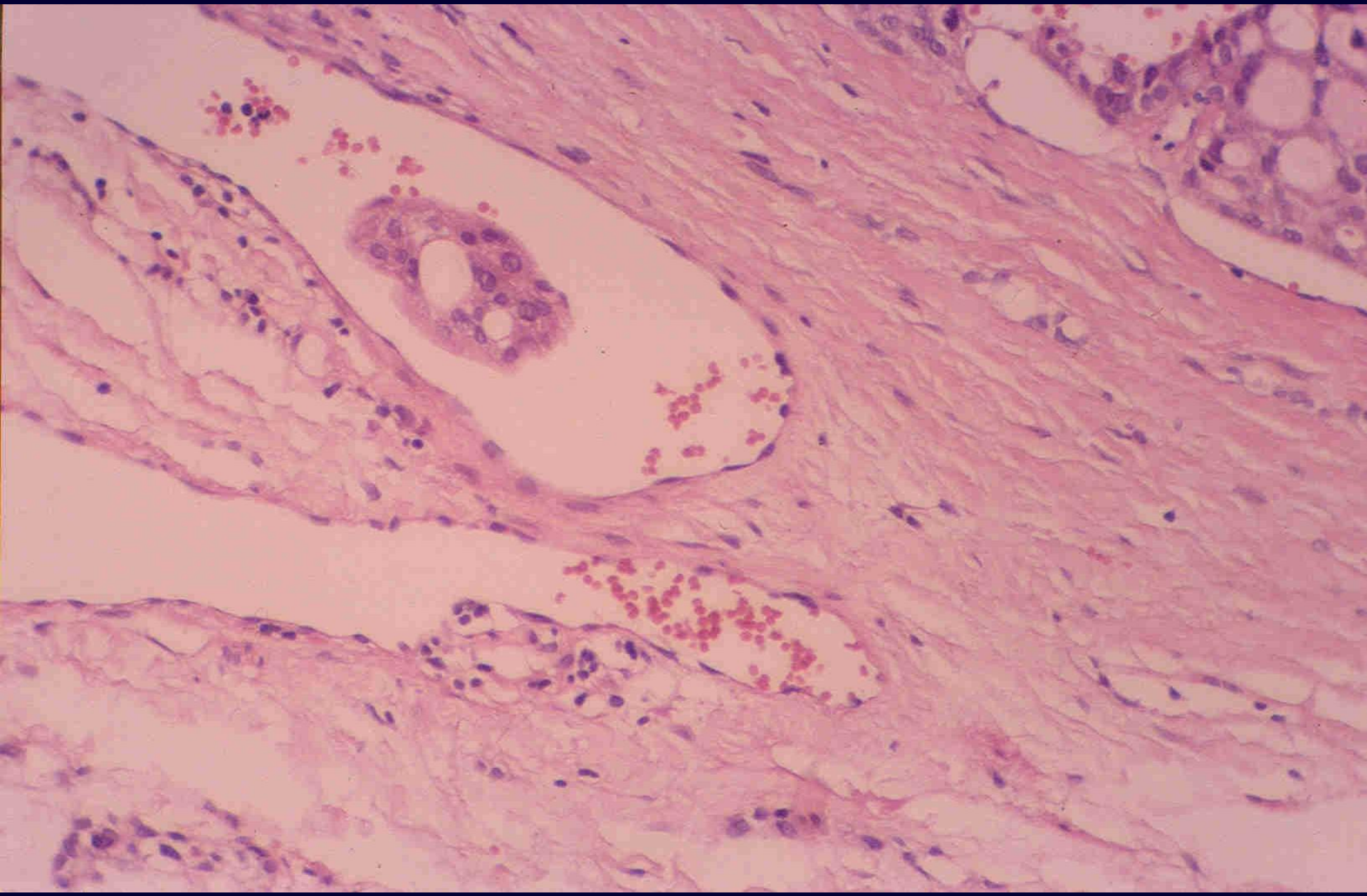


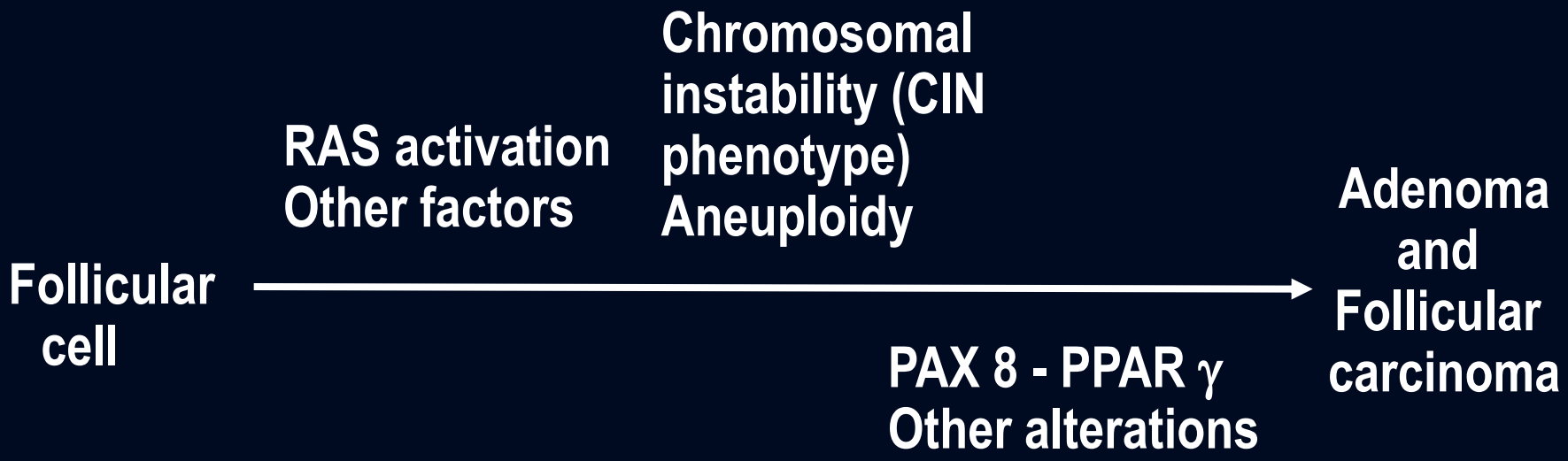



Follicular adenoma
Follicular variant of PTC
FTC
Poorly differentiated carcinoma

PITFALLS IN THE “VASCULAR INVASION” FRONT







Wiseman SM. Molecular phenotyping of thyroid tumors identifies a marker panel for differentiated thyroid cancer diagnosis. Ann Surg Oncol 15:2811, 2008

….These results suggest that further study of the molecular profile of thyroid tumors is warranted, and a diagnostic molecular marker panel may potentially improve patient selection for thyroid surgery.

A preoperative diagnostic test that distinguishes benign from malignant thyroid carcinoma based on gene expression

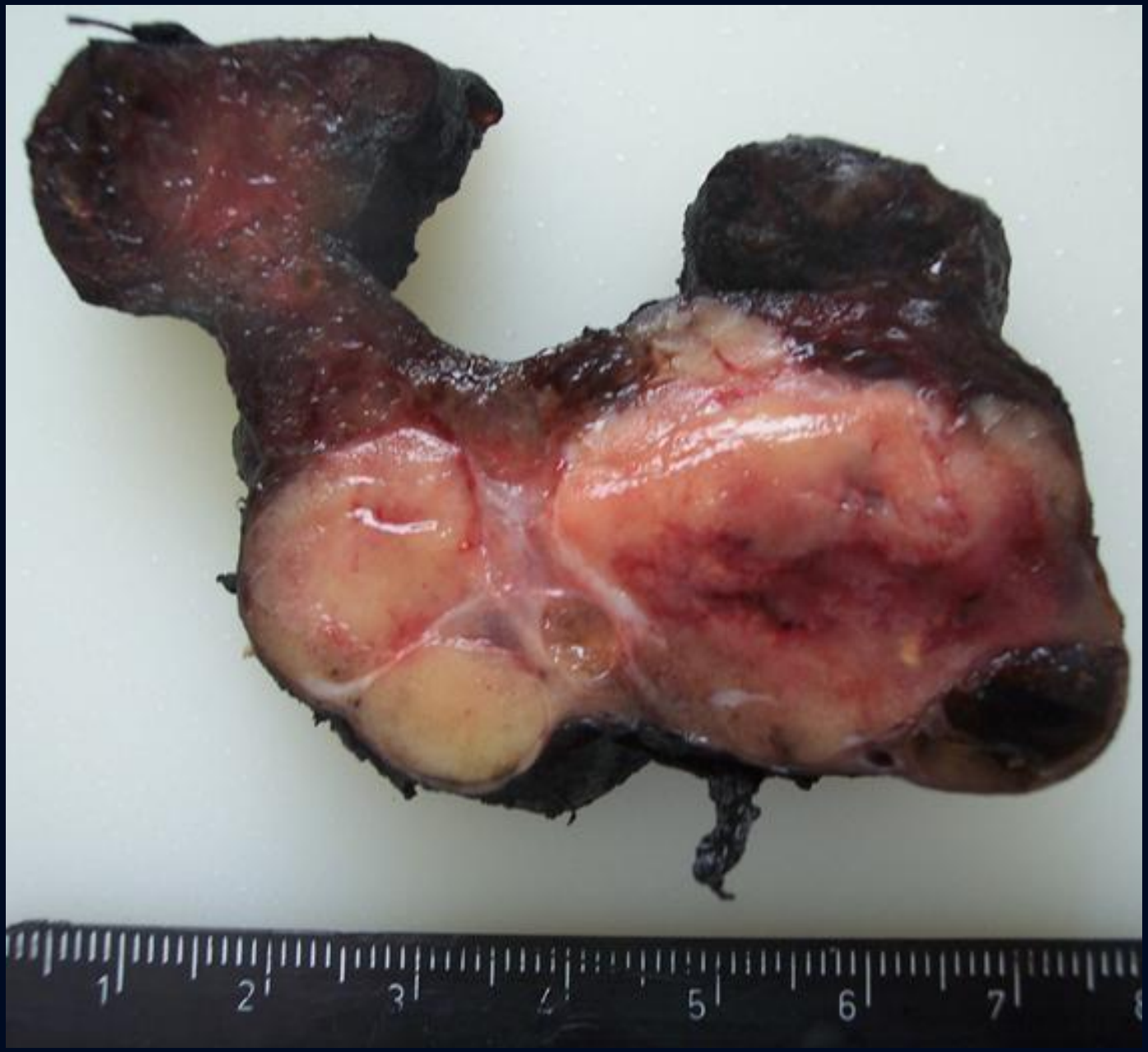
Cerutti et al, J Clin Invest 113:1234, 2004

Follicular adenoma vs follicular carcinoma

4 genes – predictive accuracy of 0.83
2 immunohistochem markers – estimated concordance of 0.76

Conclusion: A simple test based on a combination of these markers might improve preoperative diagnosis of thyroid nodules.

Widely invasive FTC



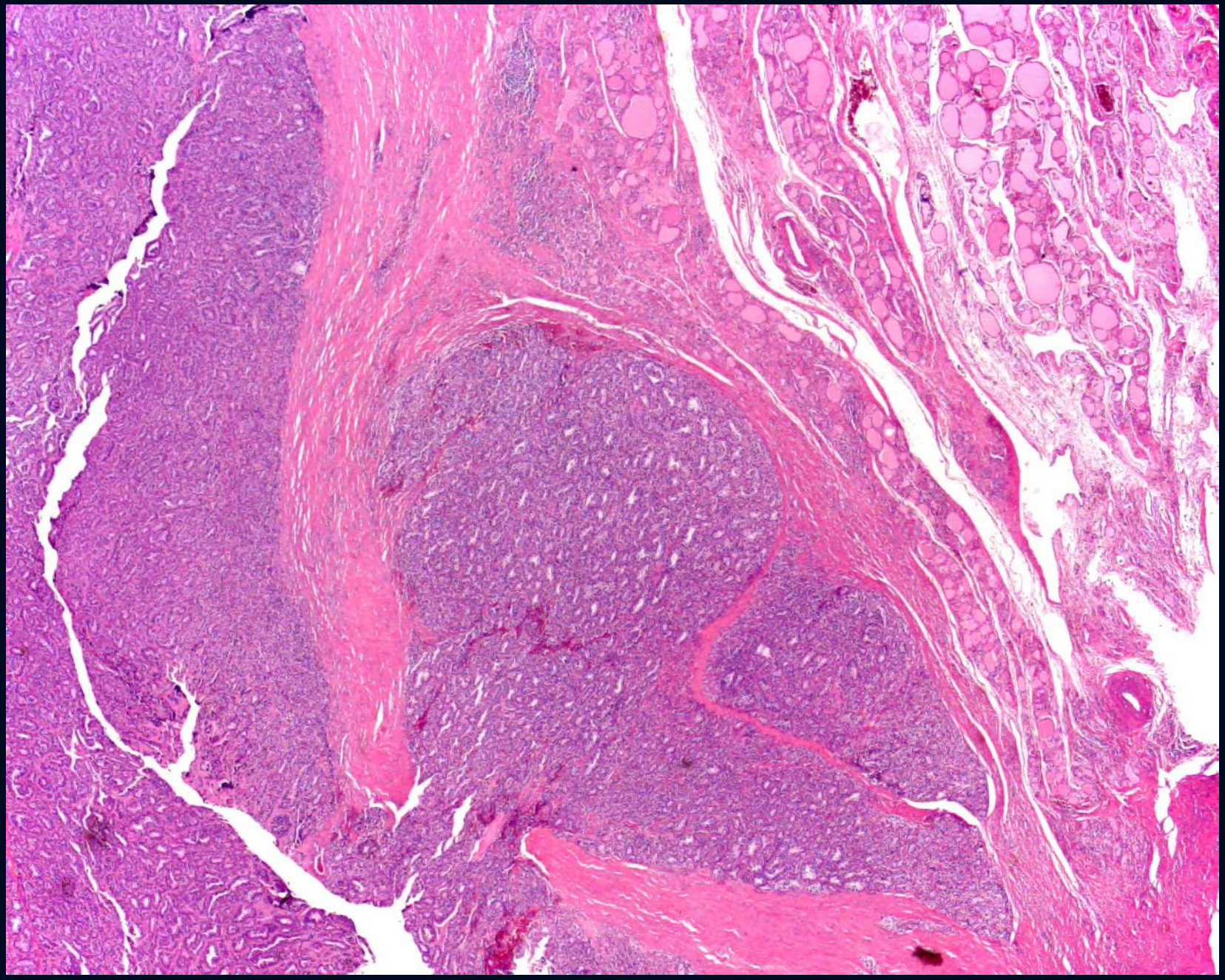


TABLE 81-1 Distribution of Cancer Incidence and Deaths for 2010

Male			Female		
Sites	%	Number	Sites	%	Number
Cancer Incidence					
Prostate	28	217,730	Breast	28	207,090
Lung	15	116,750	Lung	14	105,770
Colorectal	9	72,090	Colorectal	10	70,480
Bladder	7	52,760	Endometrial	6	43,470
Melanoma	5	38,870	Thyroid	5	33,930
Lymphoma	4	35,380	Lymphoma	4	30,160
Kidney	4	35,370	Melanoma	4	29,260
Oral cavity	3	25,420	Kidney	3	22,870
Leukemia	3	24,690	Ovary	3	21,880
Pancreas	3	21,370	Pancreas	3	21,770
All others	19	149,190	All others	20	153,260
All sites	100	789,620	All sites	100	739,940

JOHNS HOPKINS MEDICINE

Patient Care Research Education

Behind the Headlines

What's the Fastest Growing Cancer Diagnosis in U.S.?

By Simeon Margolis, M.D., Ph.D

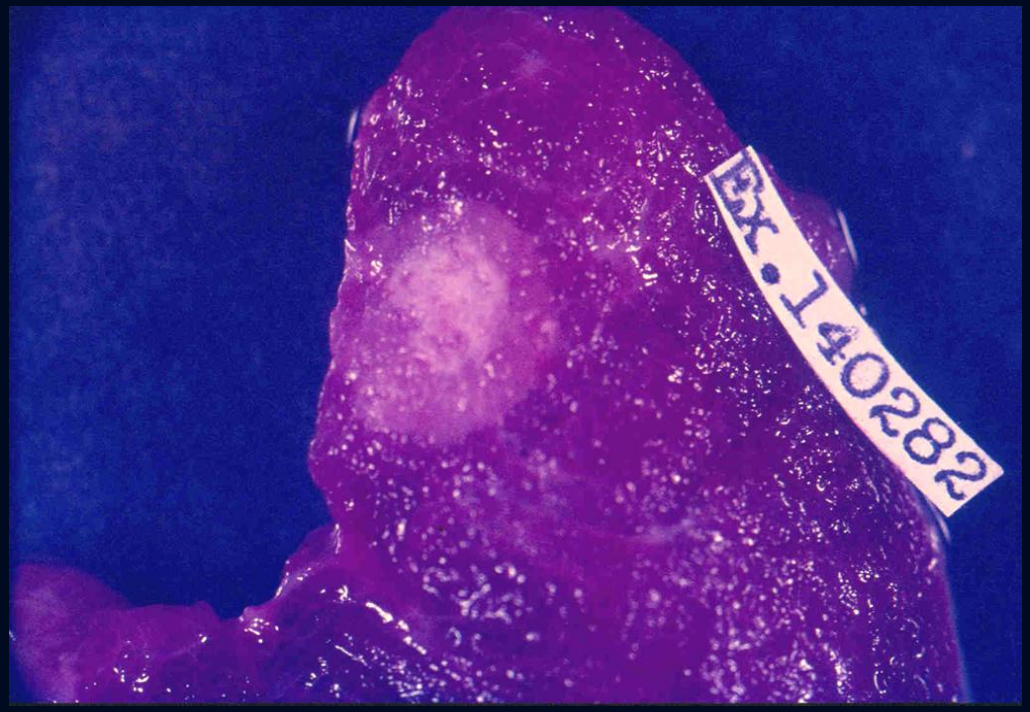
Posted Fri, Sep 05, 2008, 9:54 am PDT

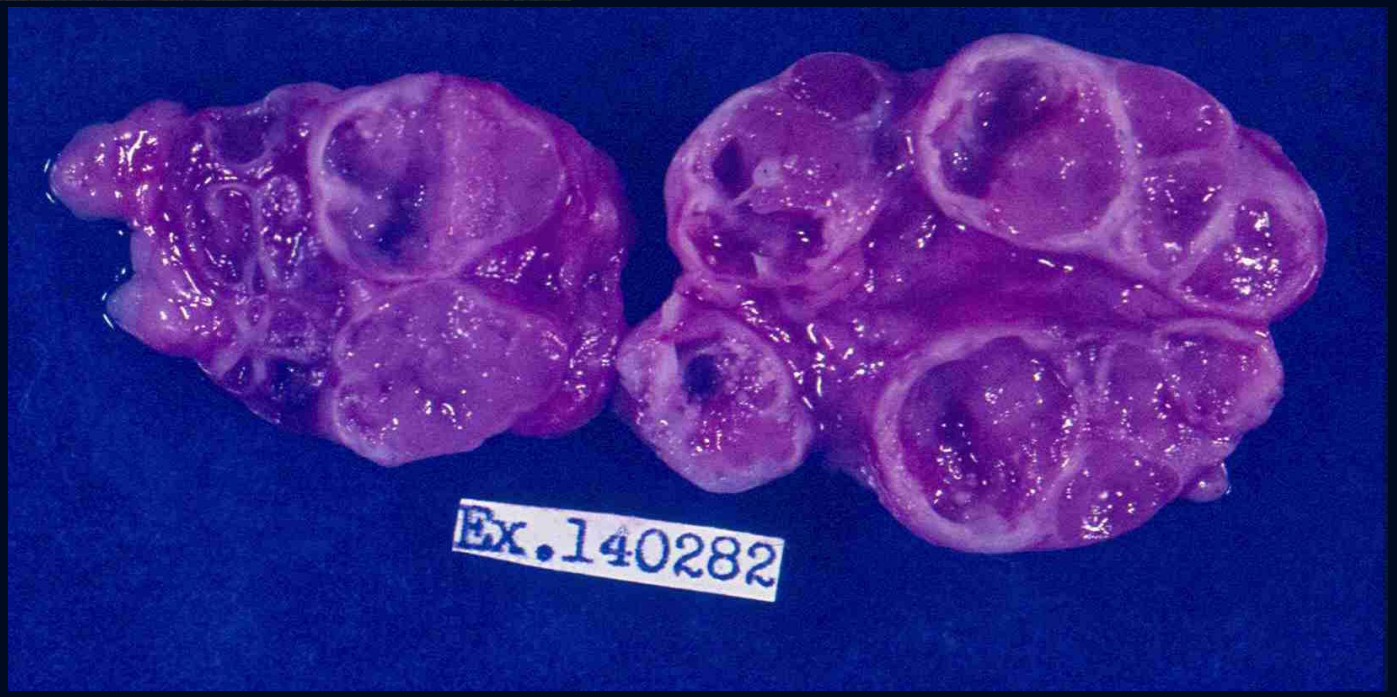
Thyroid carcinoma is the most frequent endocrine tumour

Clinically evident papillary carcinoma >80% of thyroid carcinomas

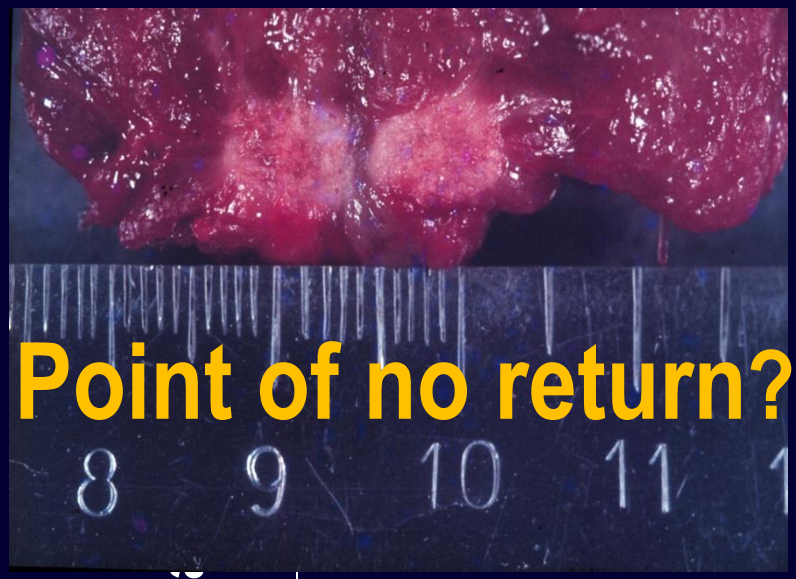
Occult papillary carcinoma (OPC) >99% of thyroid carcinomas

(1/6 of OPC have lymph node metastases)

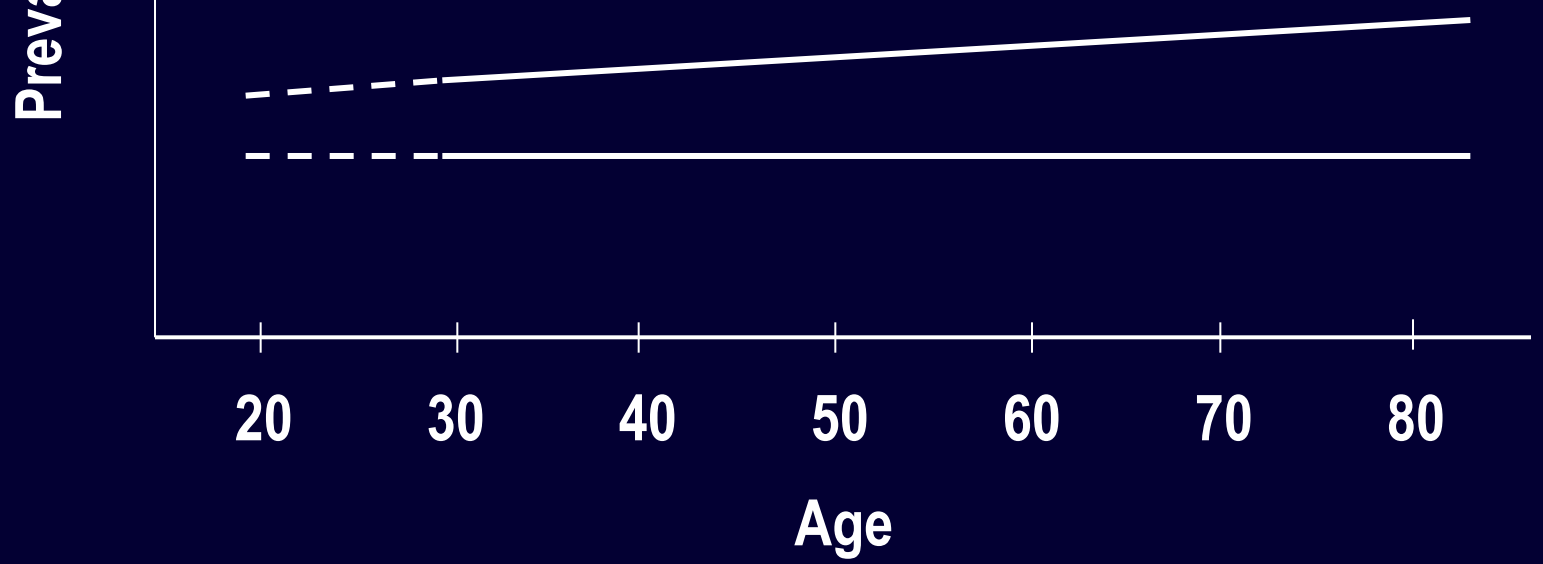

Ex.140282


Ex.140282

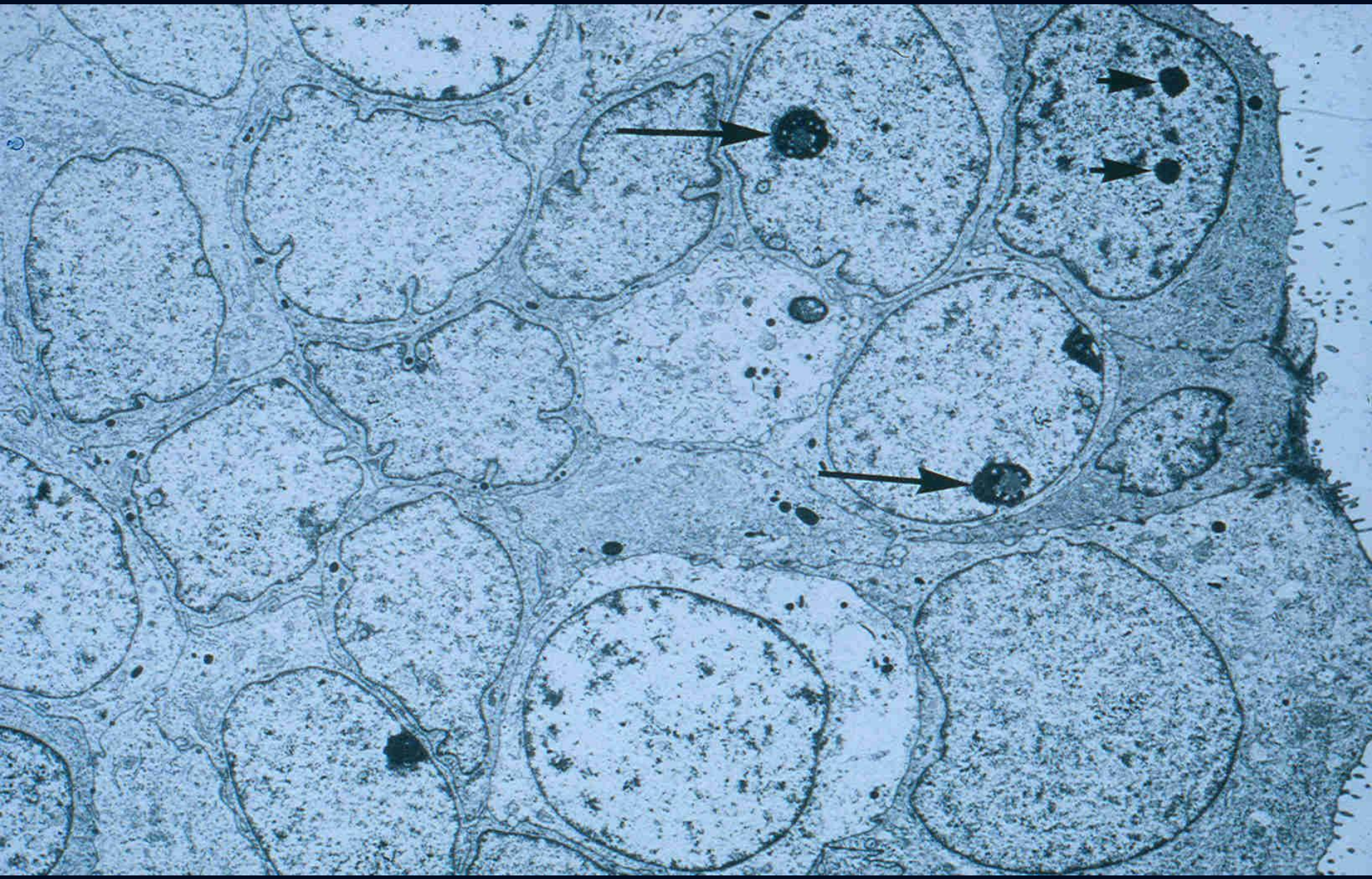
Prevalence of “occult” papillary thyroid carcinomas in autopsies



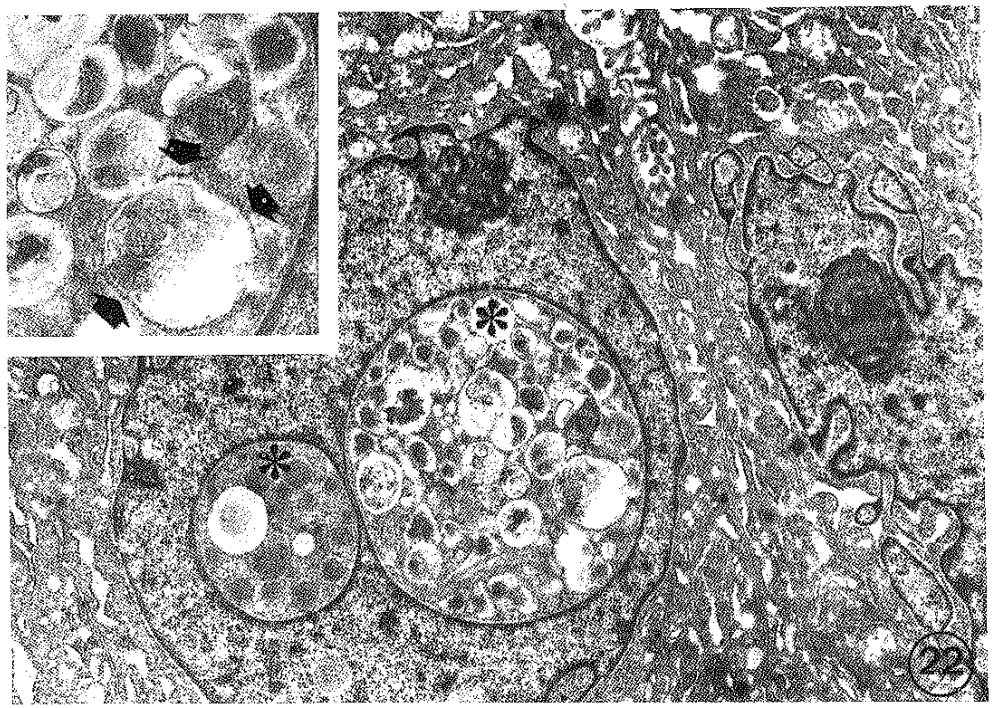
Mean values in adults
≈ 6% to ≈ 36%

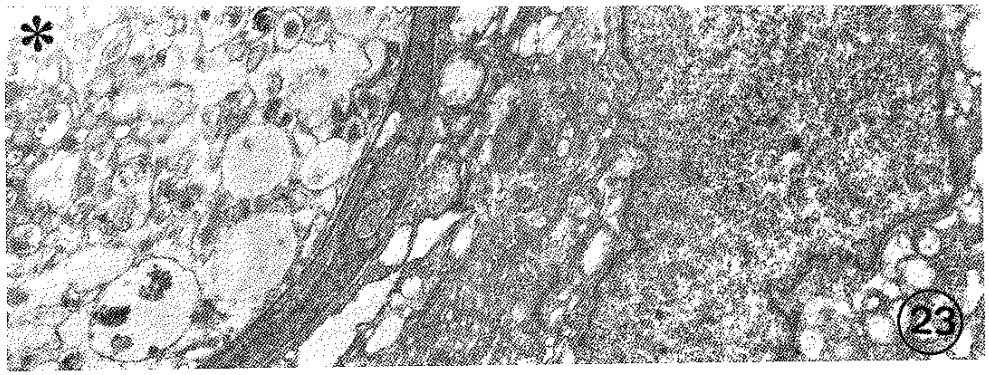


Sobrinho-Simões et al. Latent thyroid carcinoma at autopsy. A study from Oporto, Portugal. Cancer 43:1702, 1979



Sobrinho-Simões et al. Nuclear bodies and nucleolar abnormalities in PTC, Arch Pathol Lab Med 1974, 1978
Johannessen and Sobrinho-Simões. PTC have pore deficient nuclei, Int J Cancer, 1982





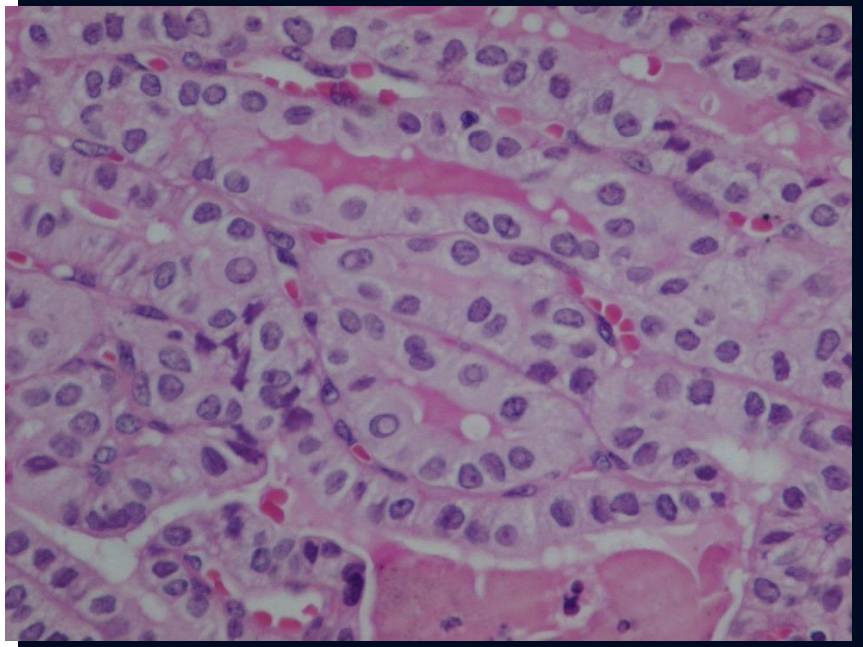
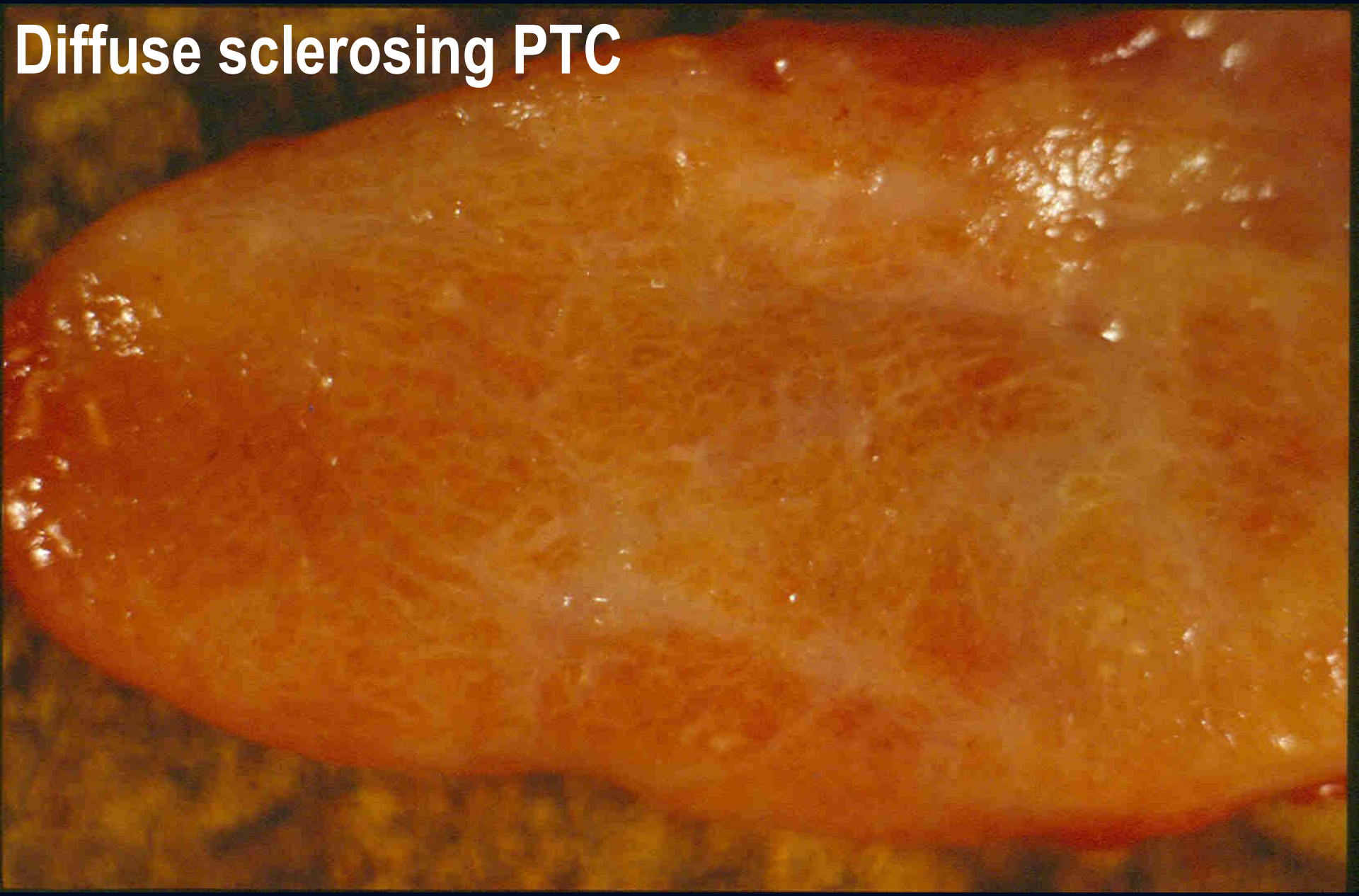


Fig. 22—As duas pseudo-inclusões nucleares (*) mostram-se preenchidas por organitos de aspecto vesicular, do tipo "corpo M", totalmente distintos dos observáveis no citoplasma. Não se observam poros na membrana nuclear que limita as pseudo-inclusões. Glutaraldeído-ósmio. × 12.000. No *pormenor,* observam-se ribossomas na superfície exterior das membranas limitantes das vesículas (setas largas). × 30.000

Fig. 23—Pseudo-inclusão nuclear com francas alterações degradativas semelhantes às observáveis nos vacúolos fagolisossómicos (*). O núcleo da célula com a pseudo-inclusão está reduzido a uma lingueta e o citoplasma mostra intensa vacuolização, em contraste com o que se observa na célula vizinha. Ósmio. × 10.000

Diffuse sclerosing PTC



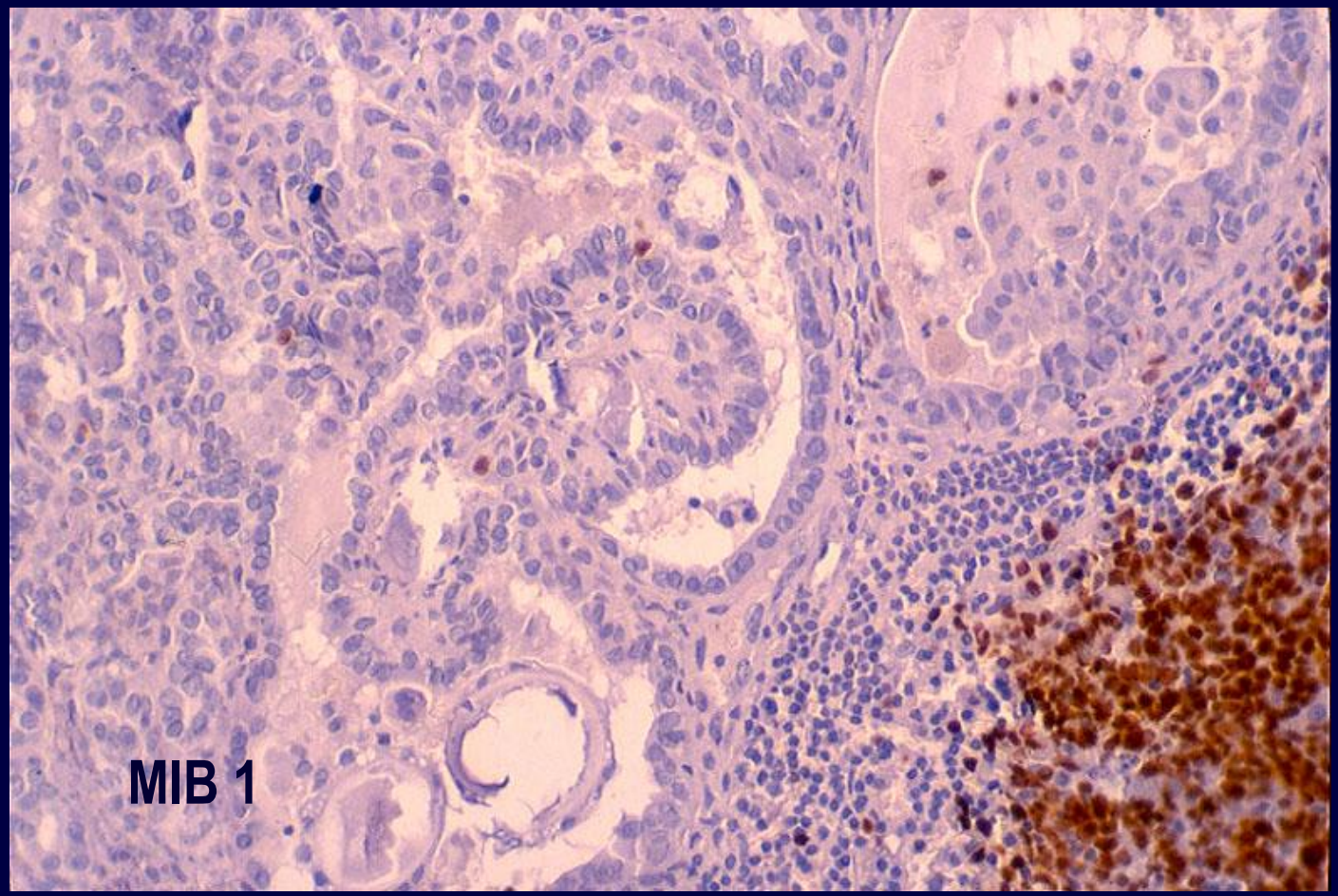
Maximal downregulation (and one inactivating mutation) of E-cadherin

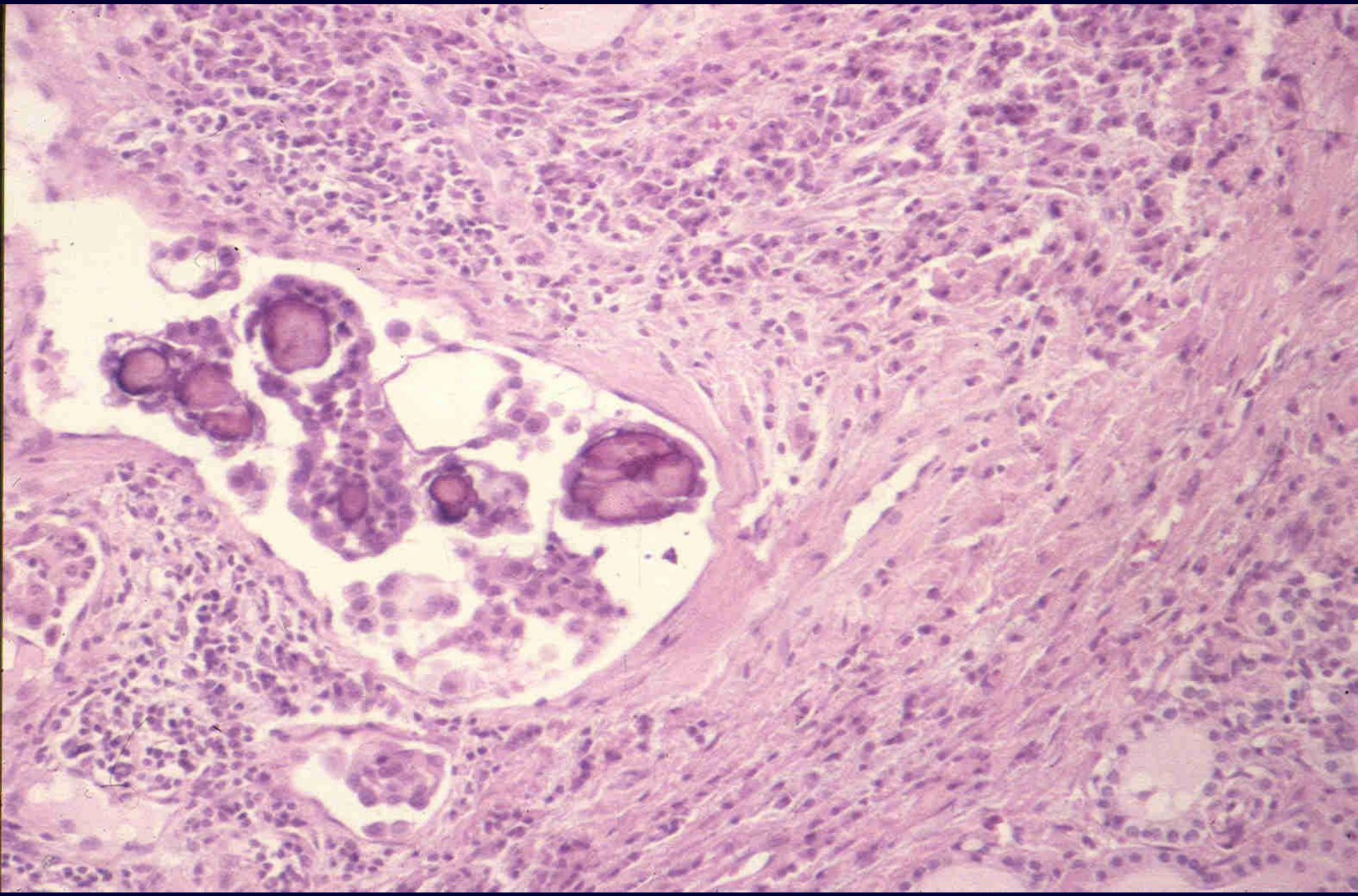
Soares et al J Pathol 194:358, 2001

A flow cytometric deoxyribonucleic acid analysis of papillary thyroid carcinoma

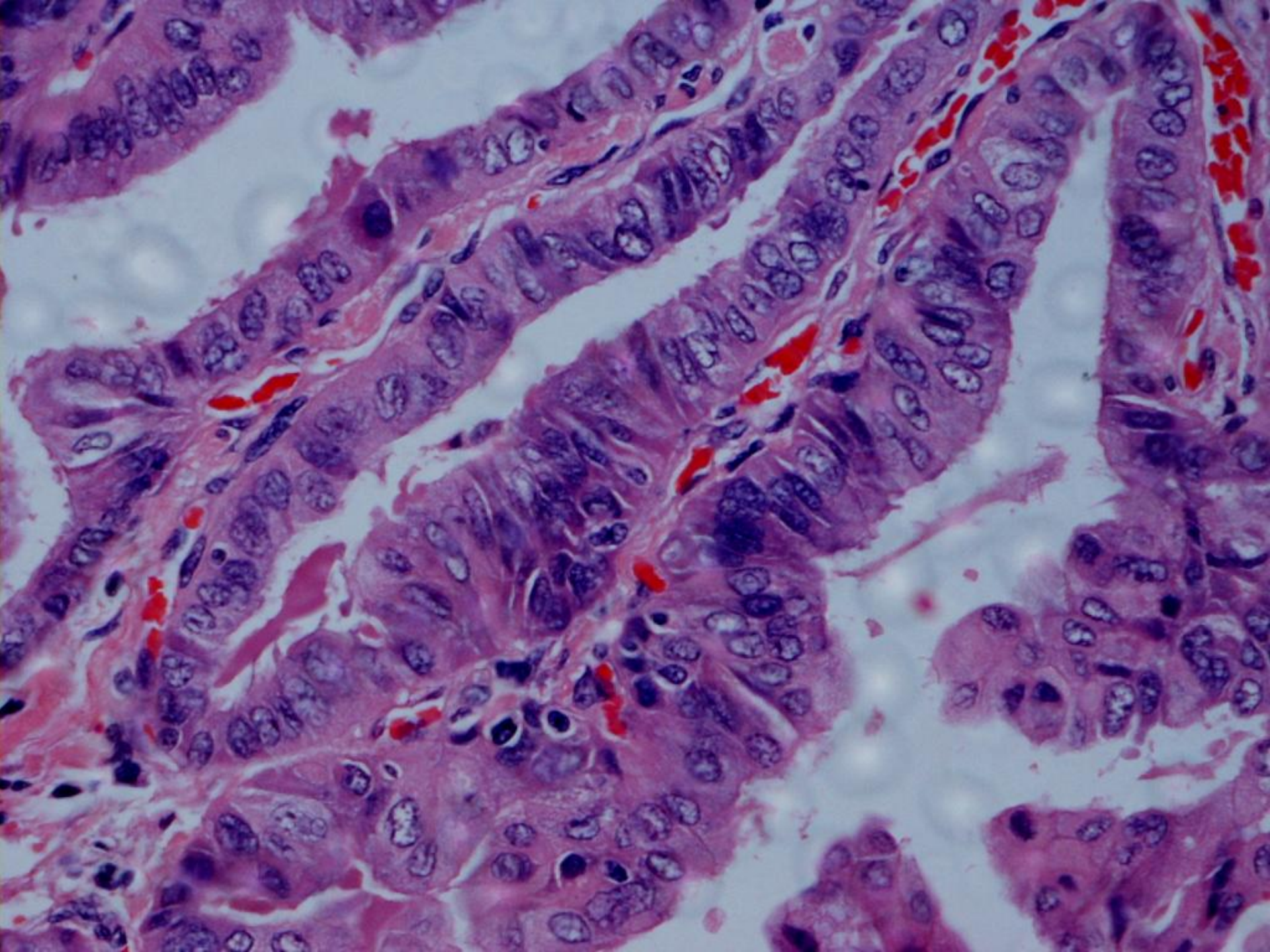
A very low percentage of S-phase cells was found both in the primary tumors and their metastases. This percentage was even lower than in normal thyroid tissue.

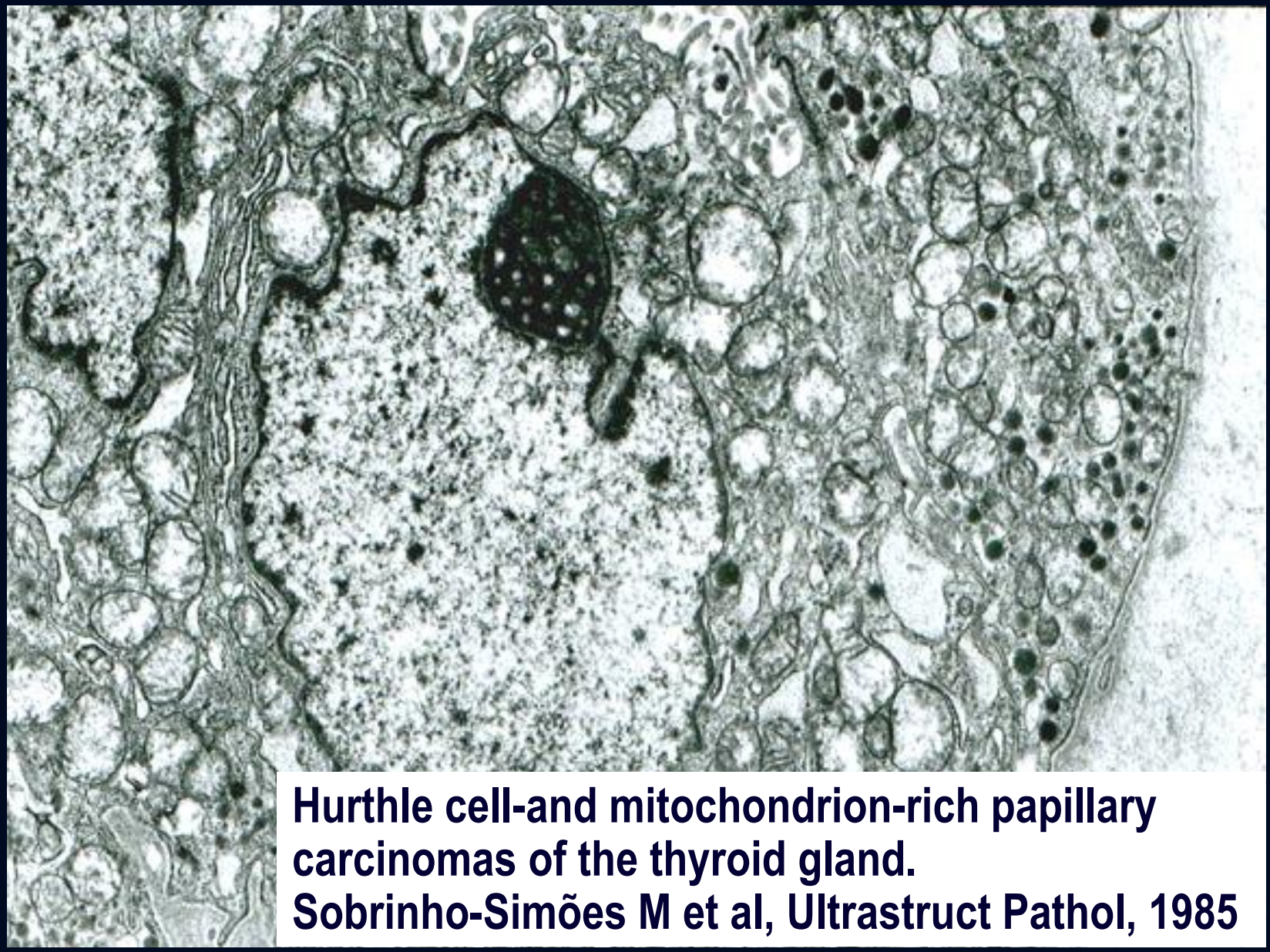
Johannessen JV, Sobrinho-Simões M, Tangen KO, Lindmo T.A. Lab Invest 45:336, 1981



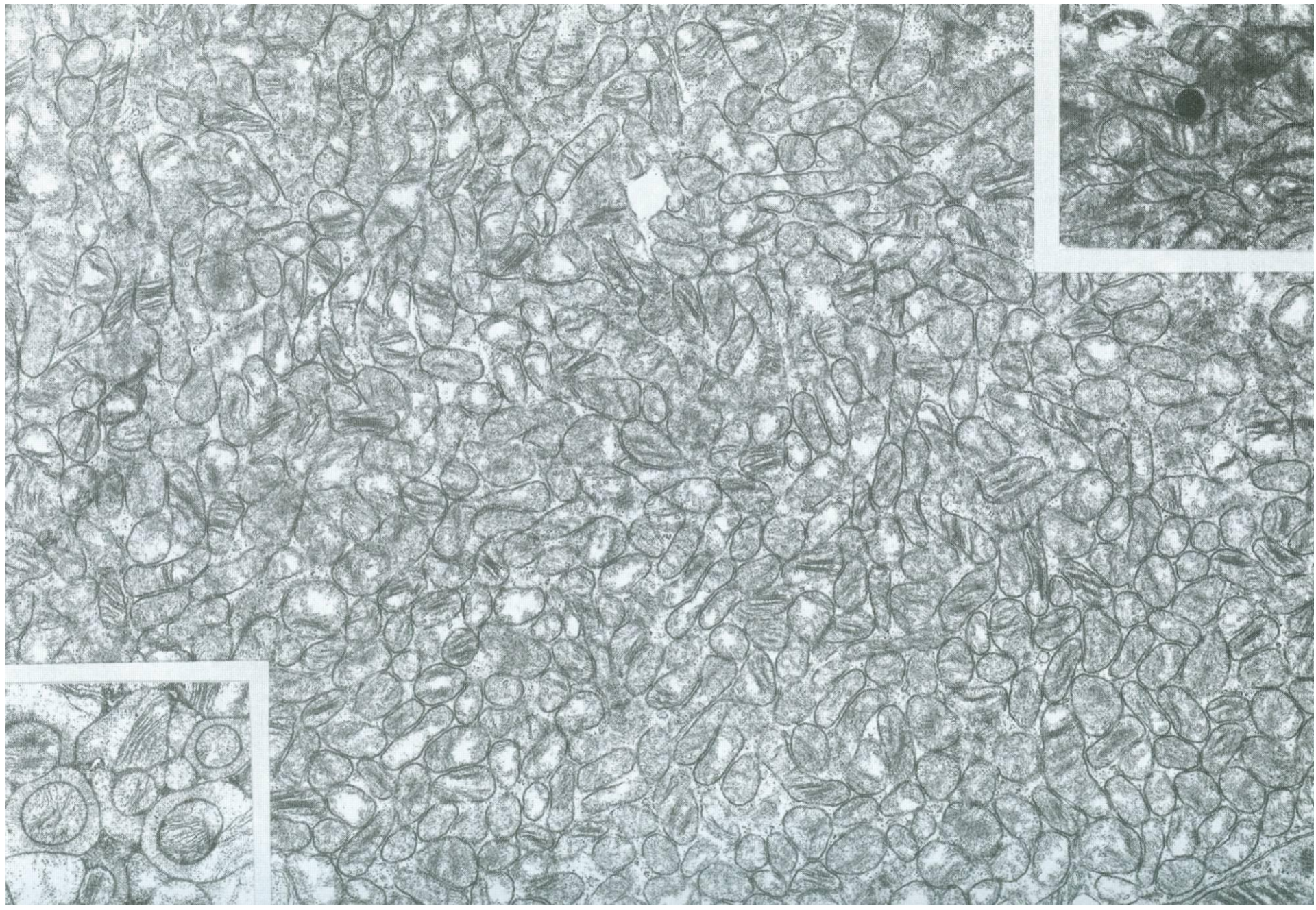


Johannessen JV, Sobrinho-Simões M. The origin and significance of thyroid psammoma bodies. Lab Invest. 43:287, 1980

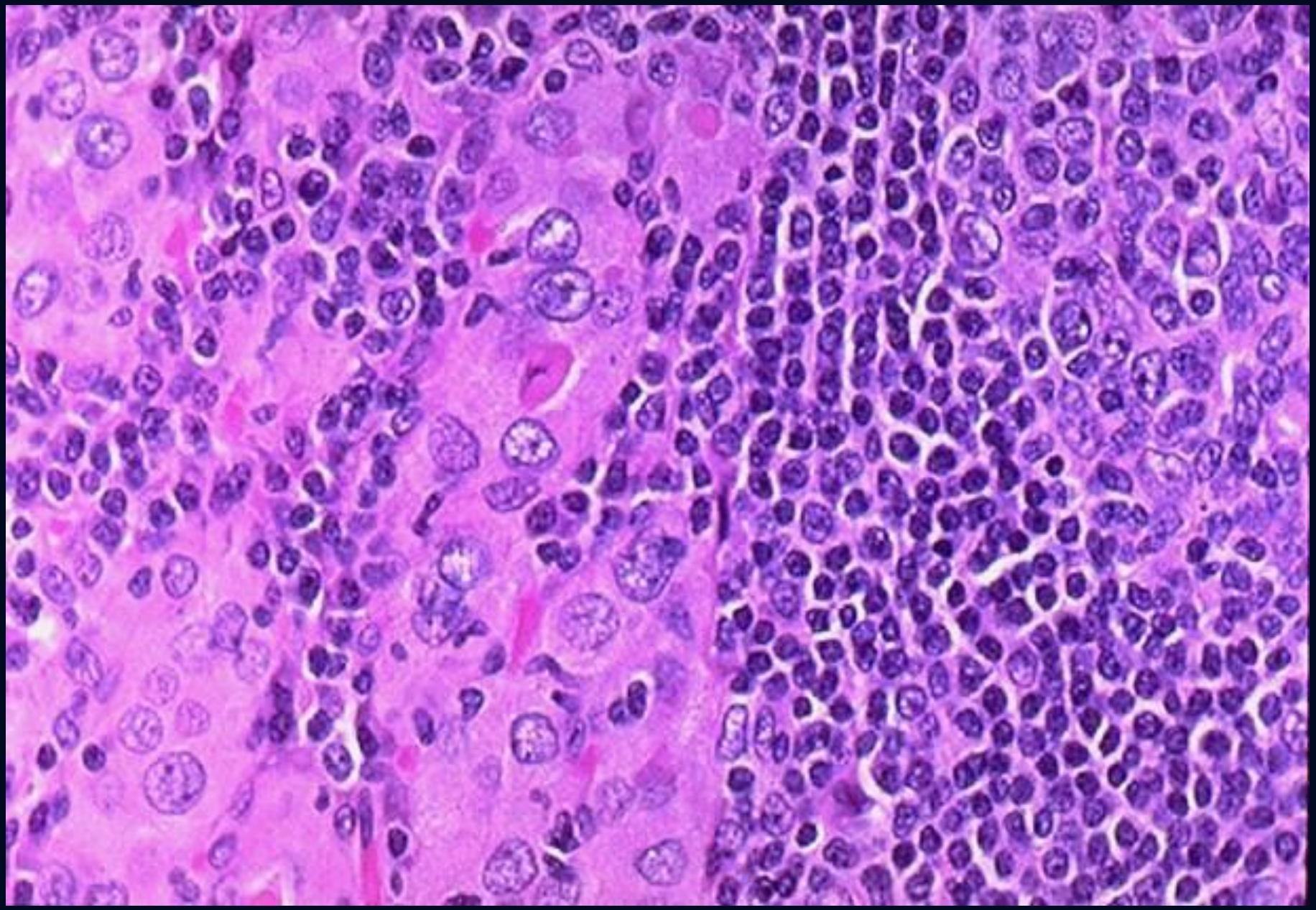




Hurthle cell-and mitochondrion-rich papillary carcinomas of the thyroid gland.
Sobrinho-Simões M et al, Ultrastruct Pathol, 1985



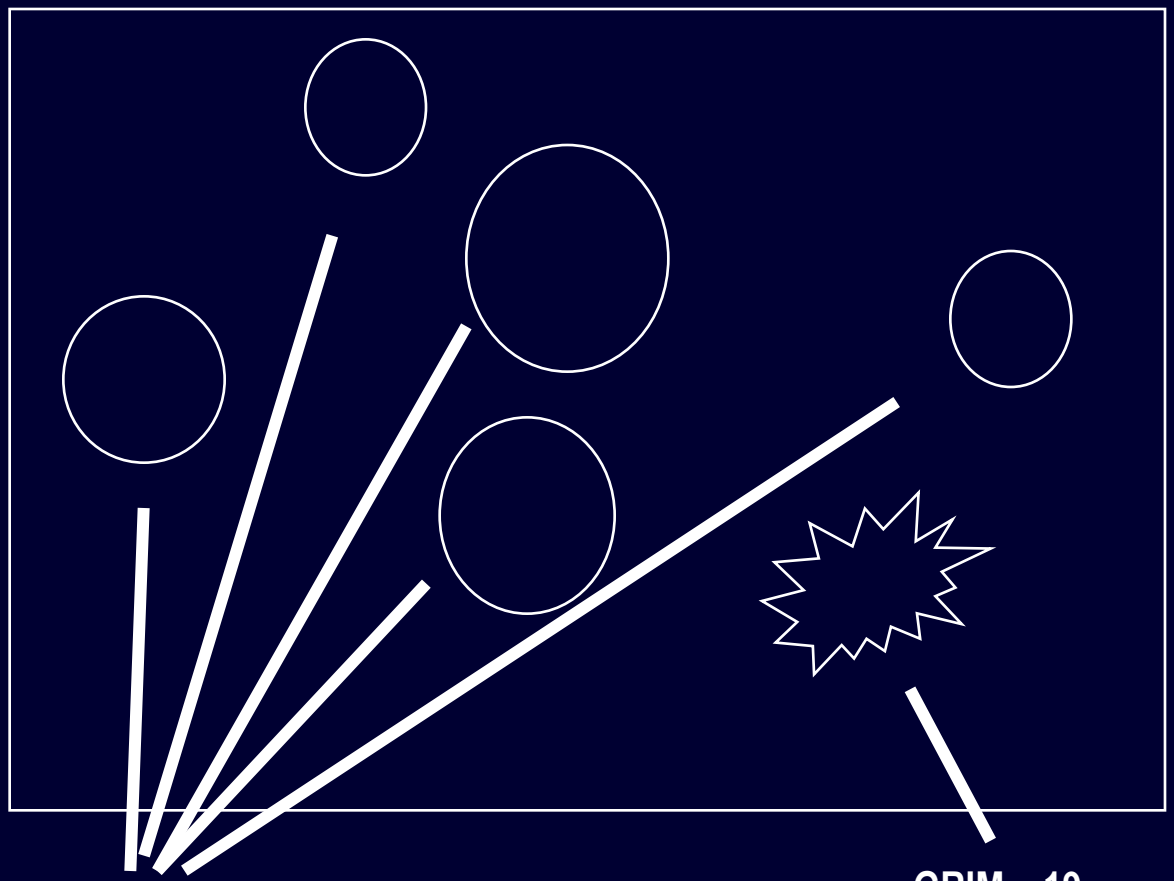
Nesland JM, Sobrinho-Simões MA et al. Hürthle-cell lesions of the thyroid: a combined study using transmission electron microscopy, scanning electron microscopy, and immunocytochemistry.Ultrastruct Pathol. 8:269, 1985



Hashimoto thyroiditis with Hürthle cells

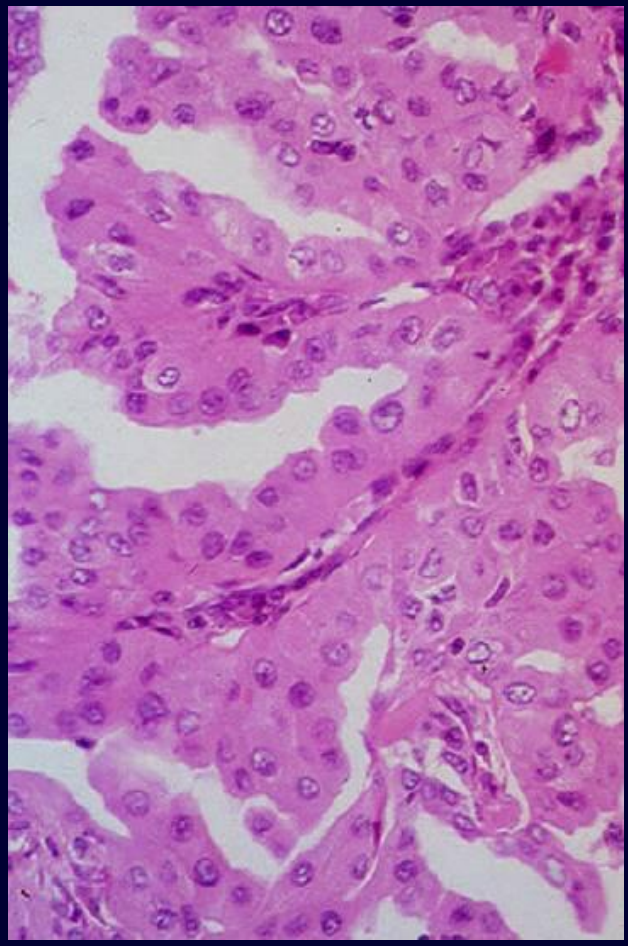
Familial nodular goiter with oncocytic features

Máximo V et al, Br J Cancer 2005



GRIM – 19
germline mutation

GRIM – 19
germline mutation
+
RET/PTC rearrangement



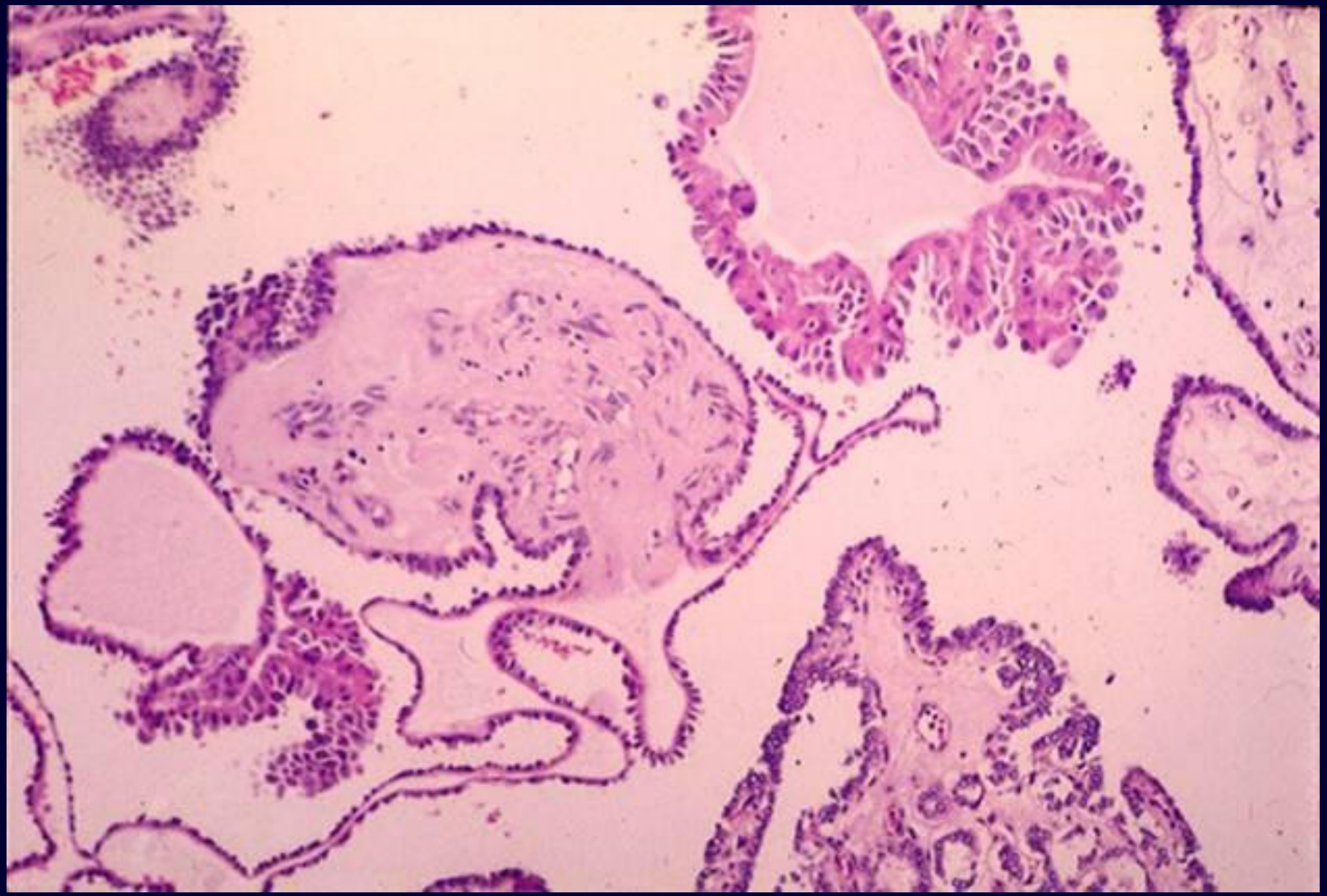
mtDNA alterations

nDNA → mt alterations

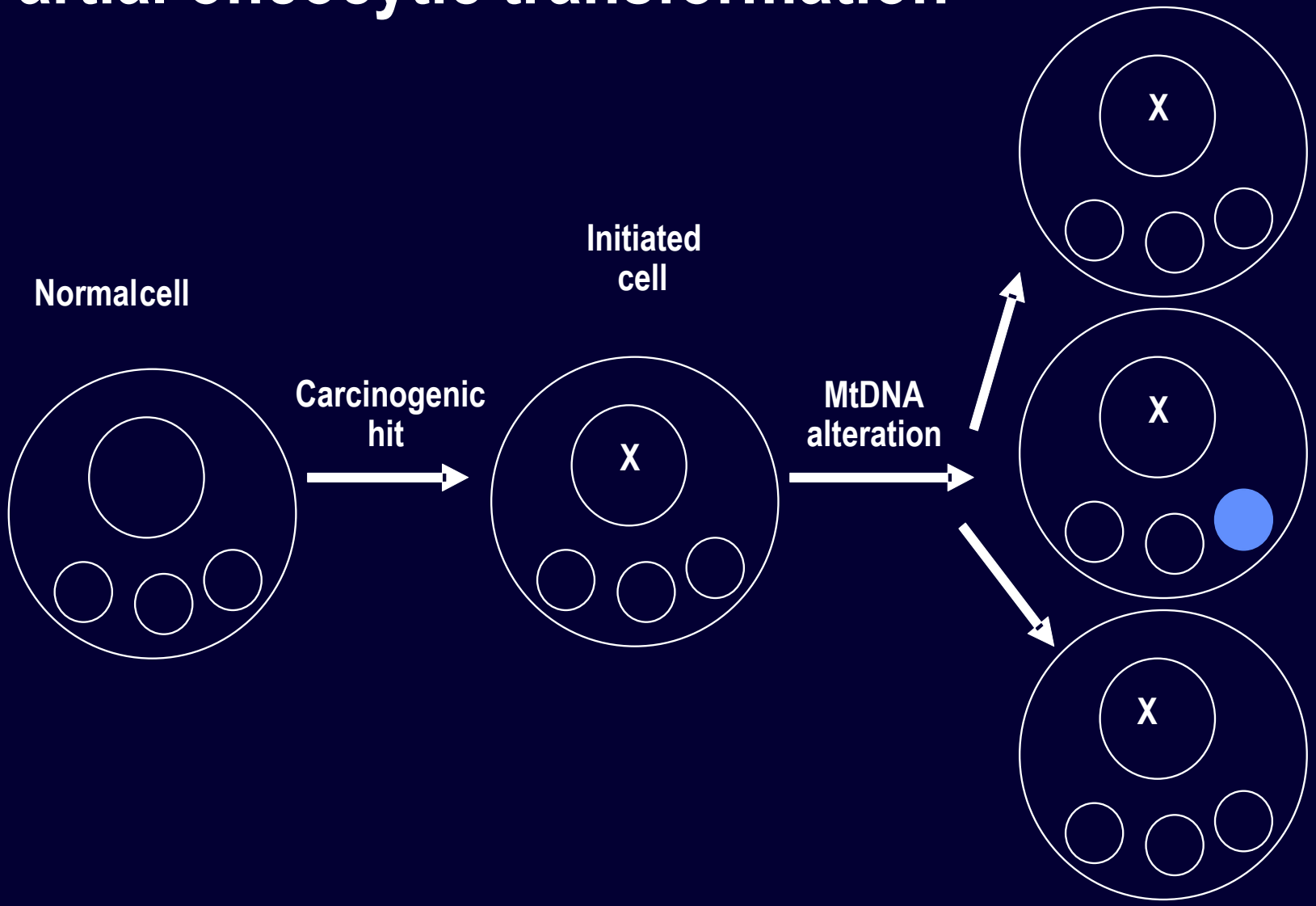
Cell growth (benign tumour) + Oncogenic step(s)

CANCER

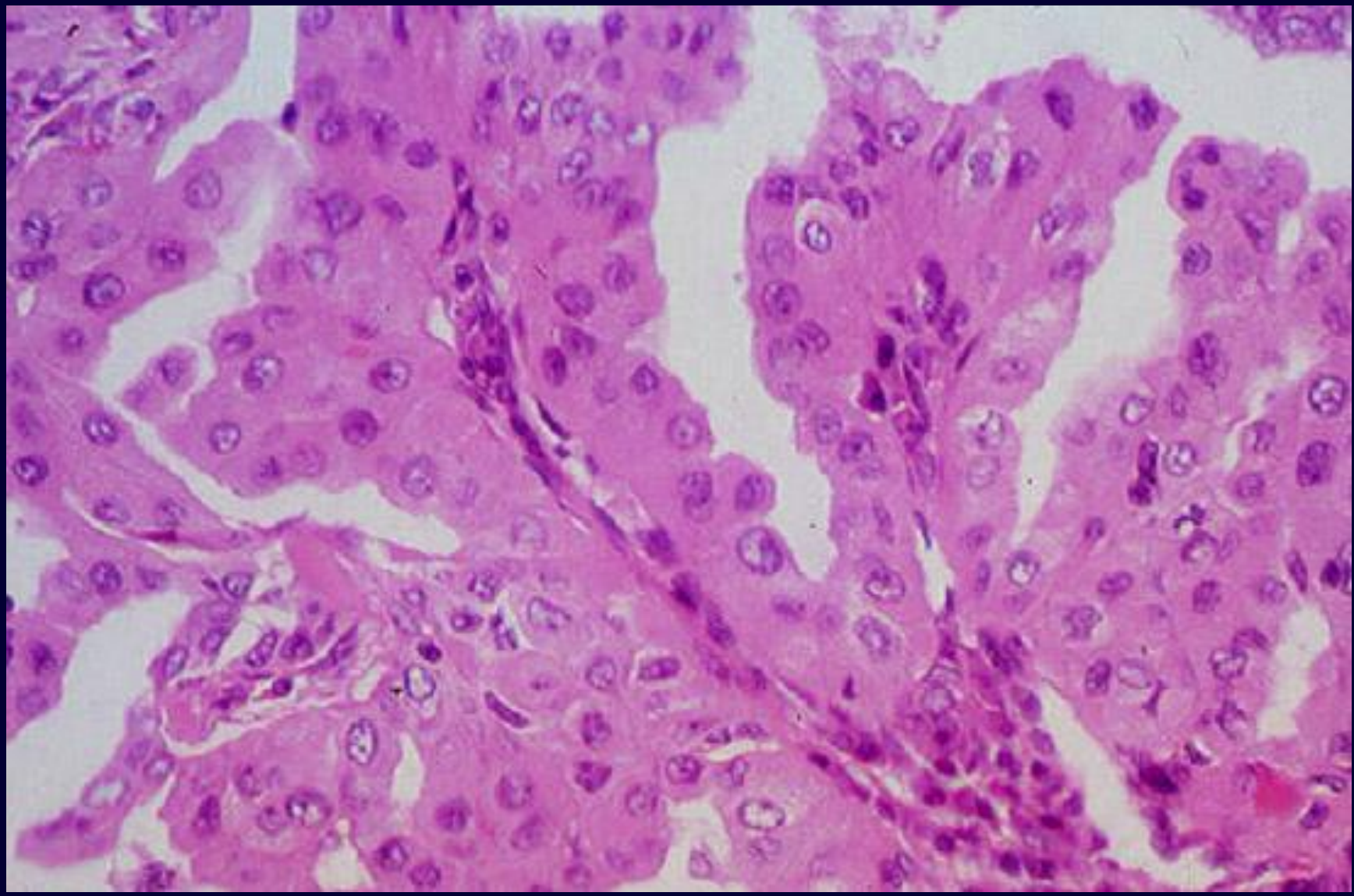
- Partial oncocytic transformation



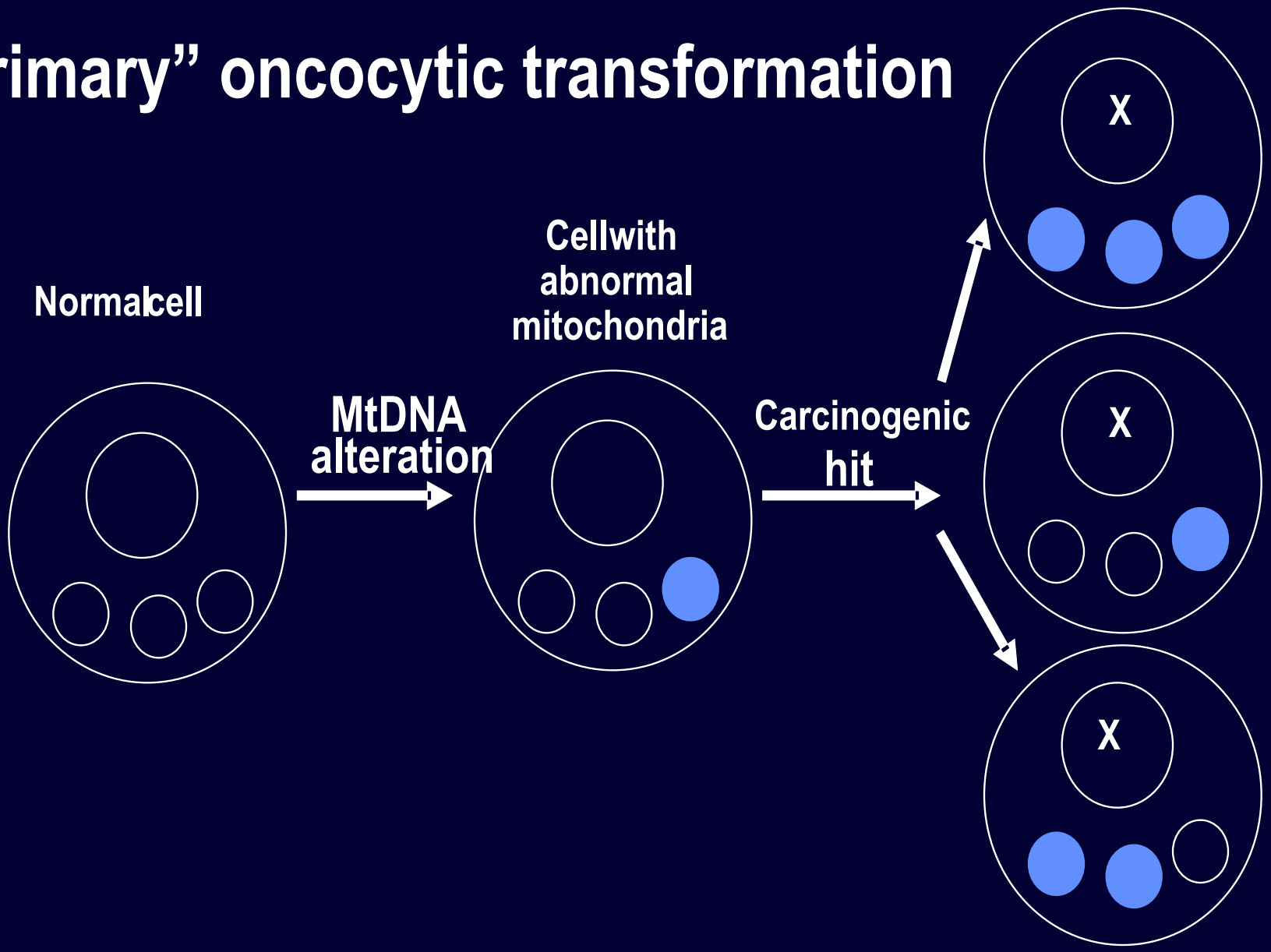
Partial oncocytic transformation



Máximo V & Sobrinho-Simões M, Virchows Arch 437:107, 2000



“Primary” oncocytic transformation



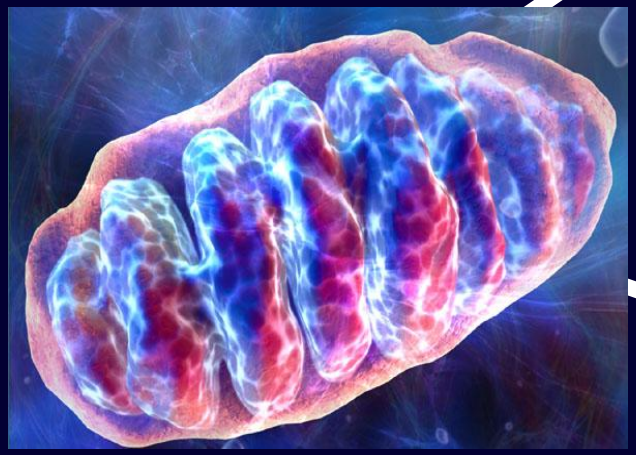
Máximo V & Sobrinho-Simões M, Virchows Arch 437:107, 2000

Oncocytic features

Nuclear genes
(GRIM-19, SDH, FH, ...)

PHENOTYPE!

Positive feedback mechanism
& mitochondrial proliferation



Lactic acidosis,
decreased apoptosis,
activation of HIF-1 α,
angiogenesis, …

Tumourigenesis

Mitochondrial genes
(Complex I, III, IV, V)

What about oncogenes and tumour supressor genes?

BRAF (V599E) MUTATION in PTC

Tumours with papillary or papillary/follicular pattern

Conventional PTC	Oncocytic PTC
53%	55%

Trovisco V et al, J Pathol 202:247, 2004
Lima J et al, JCEM 89:4267, 2004

Cheung et al.
Molecular basis of hurthle cell papillary thyroid carcinoma. J CEM 85:878, 2000

B-RAF MUTATIONS (V600E) IN PAPILLARY THYROID CARCINOMA

Cohen et al, JNCI, 2003	– 69%
Kimura et al, Cancer Res, 2003	– 36%
Soares et al, Oncogene, 2003	– 46%

Papillary carcinoma	Follicular carcinoma
Young patients	Older patients
Frequent multicentricity	Unicentricity
Diploidy or quasi-diploidy	Prominent aneuploidy
↓	↓
One (or very few) oncogenic hit(s)	Numerous oncogenic hits

Absence of genetic and chromosomal instability in papillary carcinoma
Soares P, dos Santos NR, Seruca R, Lothe RA, Sobrinho-Simões M.
Eur J Cancer. 1997 Feb;33(2):293-6.

Molecular features of papillary thyroid carcinoma

RET/PTC rearrangements (15% - 40%)
TRK rearrangements (5% - 10%)
MET overexpression (>50% ?)

.....

B-RAF mutations (30% - 50%) [0% - 75%]

Trovisco et al, J Pathol 2004
Lima et al, JCEM 89:4267, 2004

JOURNAL OF PATHOLOGY, VOL. 185: 71–78 (1998)

SPORADIC *ret*-REARRANGED PAPILLARY CARCINOMA OF THE THYROID: A SUBSET OF SLOW GROWING, LESS AGGRESSIVE THYROID NEOPLASMS?

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RET/PTC rearranged papillary carcinomas display a Bonsai phenotype

No prognostic significance – Tallini et al Clin Cancer Res 4:287, 1998

ROS, particularly H_2O_2 , can activate the MAP kinase PI3K/Akt and NFkβ pathways. ROS can also stimulate the production of MMPs and various cytokines such as TGFβ1

Reviewed in Xing. Endocrin Rel Cancer 19:C7, 2012

Since mutations in *BRAF, RAS, PIK3CA* and *PTEN* genes play a major role in the activation of such pathways, it will be interesting to see how ROS can interact or cooperate with these genetic alterations in thyroid carcinogenesis

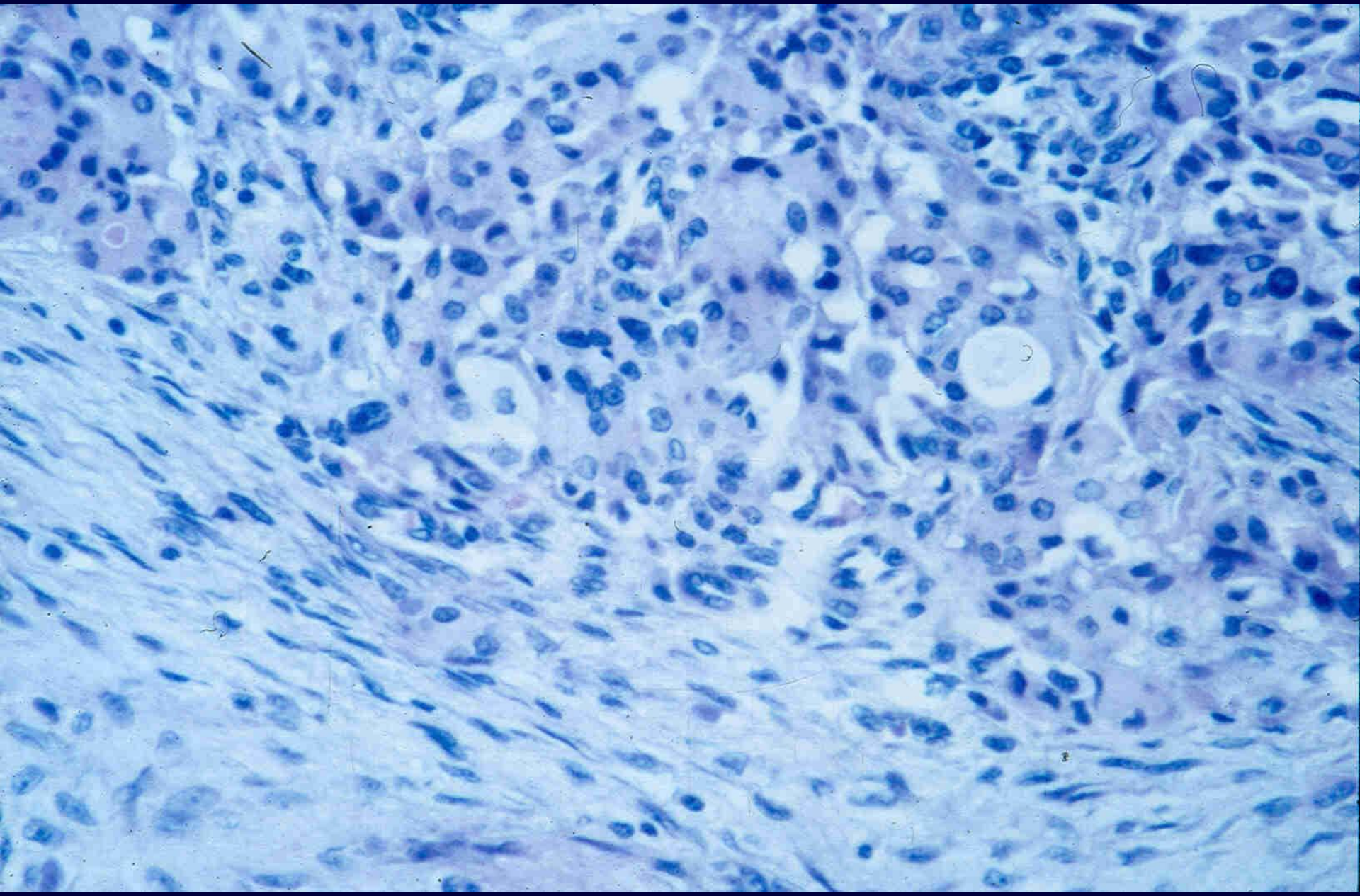
Cancer Biology Group

Progression of BRAF-induced thyroid cancer is associated with epithelial-mesenchymal transition requiring concomitant MAP kinase and TGFβ signaling.

Knauf JA et al. Oncogene 30:3153, 2011

In our hands TGFb alterations collaborate with BRAF mutations in the acquisition of an invasive phenotype

Eloy C et al. Submitted, 2012



Take home lesson

Oncobiology and Oncology

Heterogeneity

Topography

Cell and nuclear morphology

Chronology (time factor)

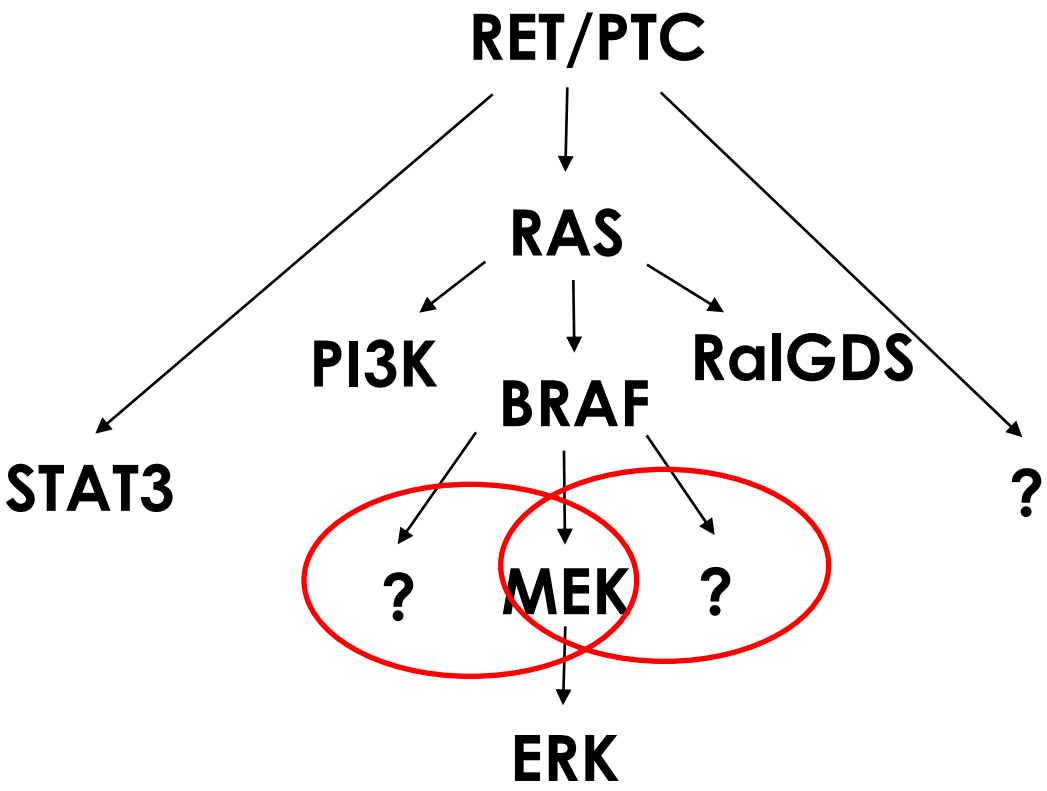
Back to the old major genes: P53, Rb, RAS,…

Back to ploidy status

Back to TIL's and TAM's (tumour lymphocytes and macrophages)

Back to……

RET/PTC-RAS-RAF-MEK-ERK?

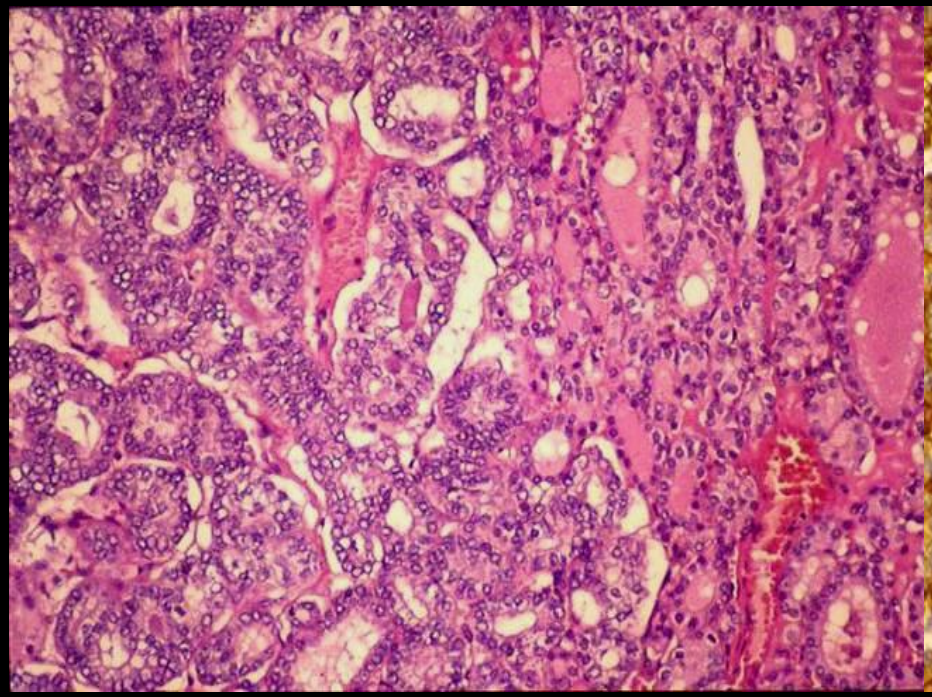


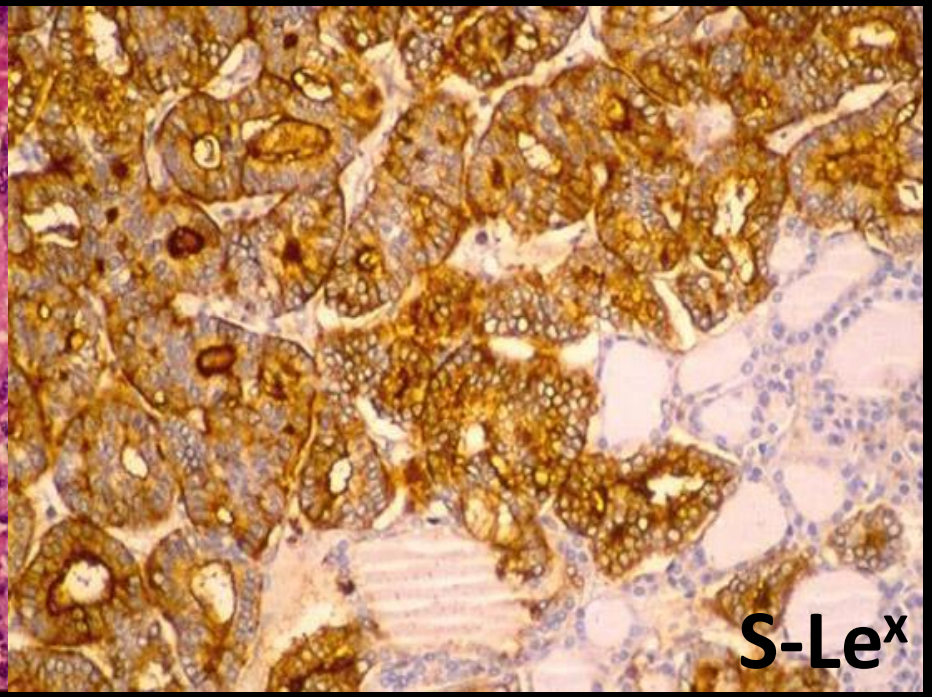
Immunohistochemical markers used in PTC diagnosis

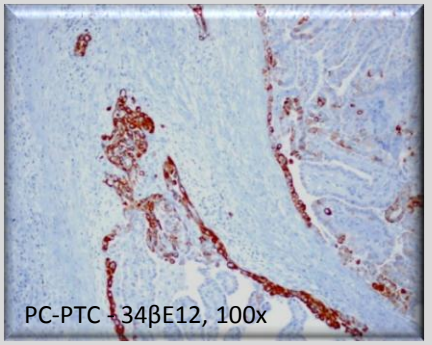
Cytokeratin 19
Lewis X and S Lewis X
Galectin 3
HBME1
Fibronectin 1
.....

Galectin-3 and HBME-1 expression in well-differentiated thyroid tumors with follicular architecture of uncertain malignant potential

Mauro Papotti[1], Jaime Rodriguez[2], Roberta De Pompa[1], Armando Bartolazzi[3] and Juan Rosai[2]







Morphological and epigenetic alterations at the invasive front of the tumours

The epithelial-to-mesenchymal transition (EMT) phenotype in PTC

- ↓ polarity/cohesiveness
- ↑ spindle cell shape
- ↓ expression of beta-catenin and E-cadherin at the cell membrane
- ↑ nuclear expression of STAT3
- ↑ increased nuclear expression of cyclin D1
- ↑ cytoplasmatic expression of vimentin, fibronectin1, met, ret, CK 5/6, galectin-3 and **TGFβ**

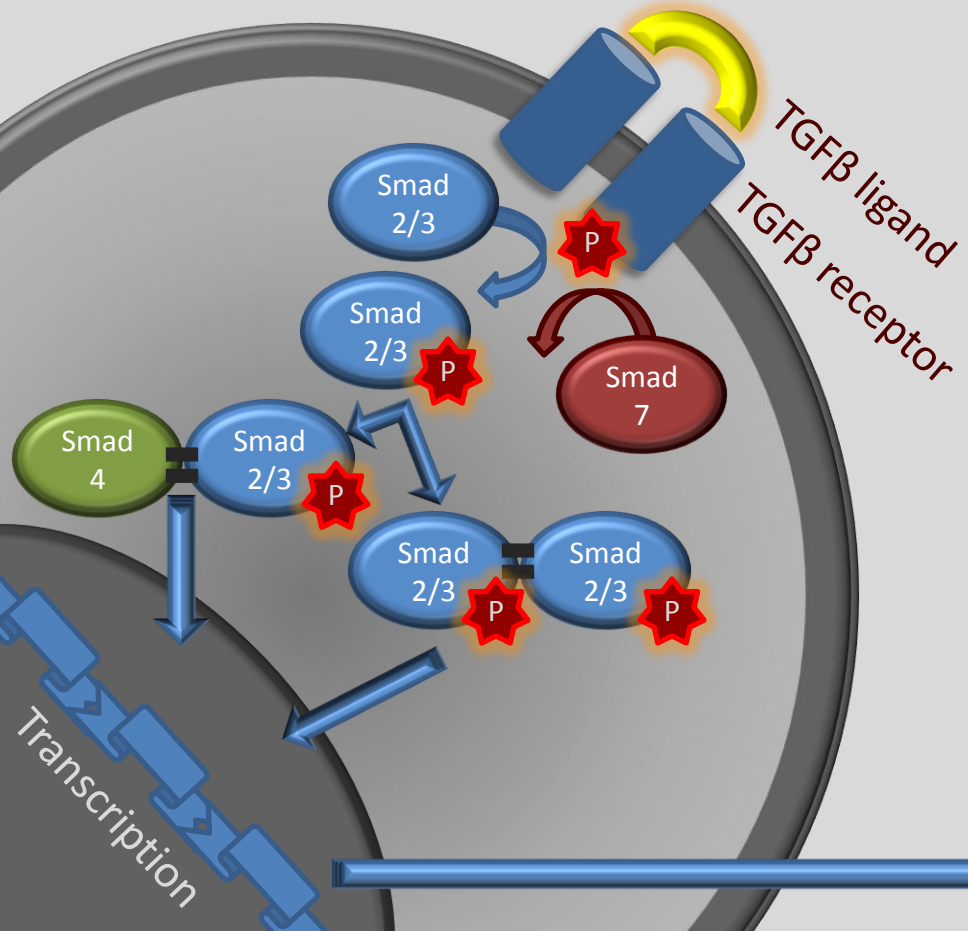
Cross-talk between tumour cells and cellular (and non-cellular) elements of the stroma

- ↓ dendritic cells and lymphocytes at the periphery of PTCs with distant metastases

- ↑ tumour-associated macrophages in poorly differentiated carcinomas correlate with invasion and decreased survival

Fonseca E *et al* 1997, Fiumara A *et al* 1997, Herrmann ME *et al* 2002, Scarpino S *et al* 2004, Vasko V *et al* 2007, Ryder M *et al* 2008, Jung CK *et al* 2010, Liu Z *et al* 2011

TGFβ/Smad dependent pathway



TUMOUR SUPPRESSION

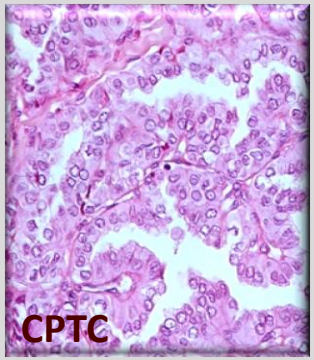
Growth-suppressive effects in normal thyroid and PTC cell lines

TUMOURIGENESIS

TGFβ suppressive effects can be abrogated at EMT allowing the development of pro-oncogenic effects, namely through the inhibition of cell adhesion and enhancement of motility

Bravo SB *et al* 2003,Matsuo S *et al* 2006, Riesco-Eizaguirre *et al* 2009

Molecular alterations and clinical behaviour



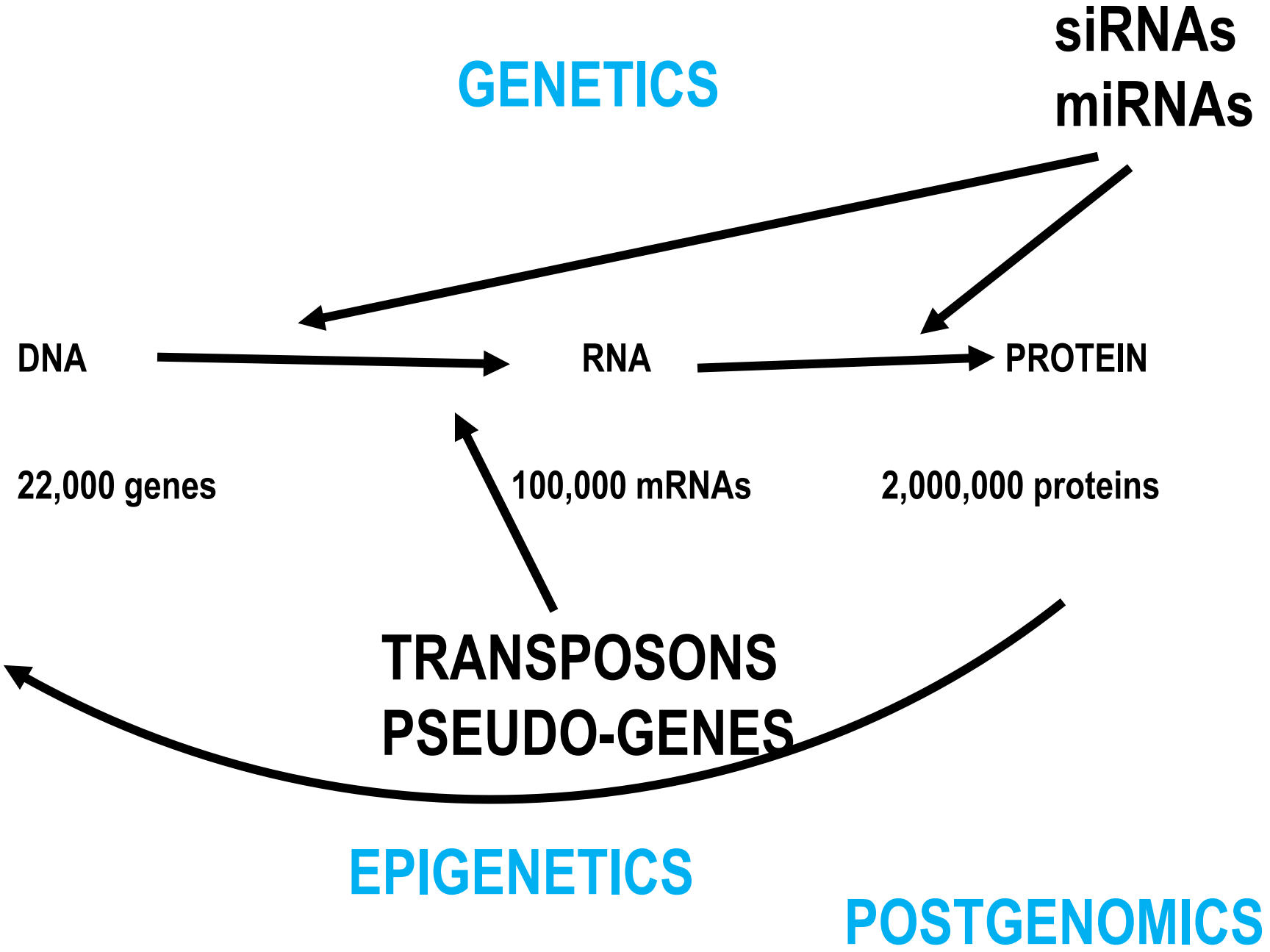
BRAF (V600E) mutations: 40-54%
RAS mutations: 0-23%
RET/PTC rearrangements: ~40%
PAX8/PPARγ rearrangements: 0%

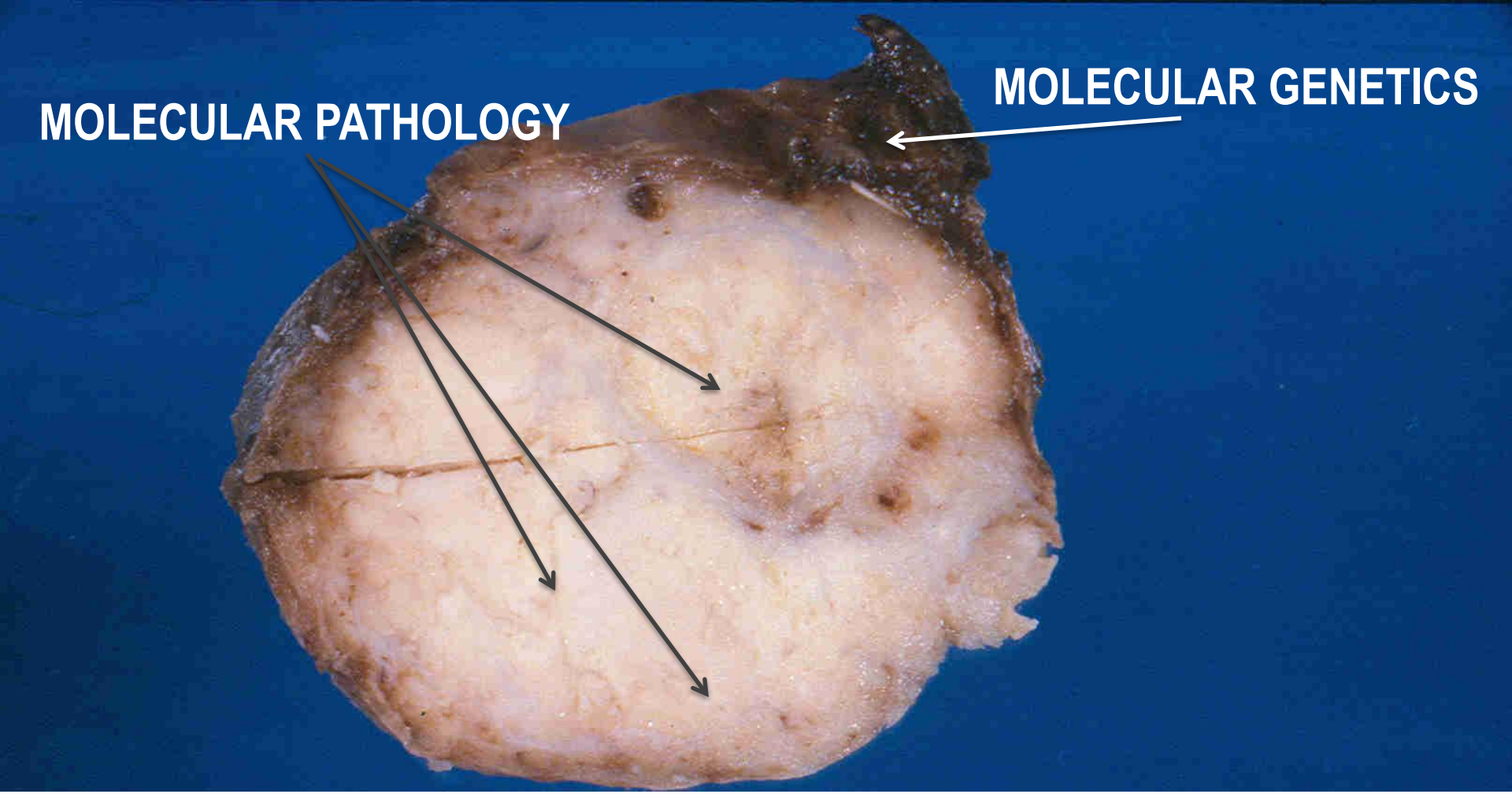
BRAF (K601E) mutations: 7-27%
RAS mutations: 25-43%
RET/PTC rearrangements: ~10%
PAX8/PPAR γ rearrangements: ~10%

BRAF (V600E) mutations: 7-83%
RAS (*N-RAS* Q61R) mutations: 0-43%

MAPK pathway elements

Sobrinho-Simões M et al. *Int J Surg Pathol* 2010; 18: 209S-212S *(PAPER 4)*


GENETICS
siRNAs
miRNAs
DNA
RNA
PROTEIN
22,000 genes
100,000 mRNAs
2,000,000 proteins
TRANSPOSONS
PSEUDO-GENES
EPIGENETICS
POSTGENOMICS



MOLECULAR MEDICINE

MOLECULAR GENETICS - germline DNA alterations

MOLECULAR PATHOLOGY - somatic, non-hereditary genetic alterations

Sobrinho-Simões M, American Board of Pathology, S. Francisco, 2001

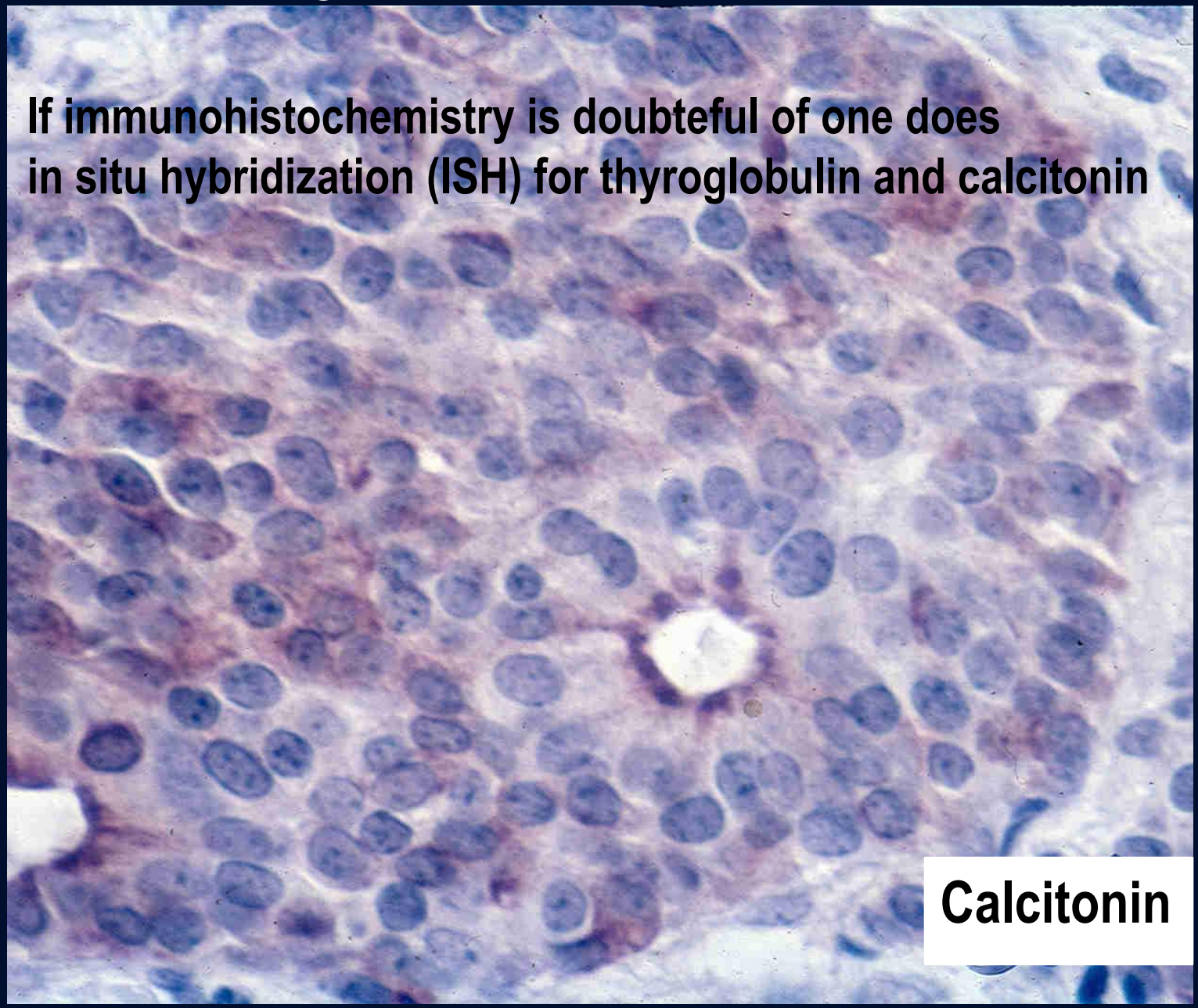
Oncogene Addiction

I. Bernard Weinstein[1,2] and Andrew Joe[1,2]

Abstract

Cancer cells contain multiple genetic and epigenetic abnormalities. Despite this complexity, their growth and survival can often be impaired by the inactivation of a single oncogene. This phenomenon, called "oncogene addiction," provides a rationale for molecular targeted therapy. The efficacy of this strategy requires novel methods, including integrative genomics and systems biology, to identify the state of oncogene addiction (i.e., the "Achilles heel") in specific cancers. Combination therapy may also be required to prevent the escape of cancers from a given state of oncogene addiction. [Cancer Res 2008;68(9):3077–80]

Medullary carcinoma



Major problems in thyroid oncology

- Separation of follicular cell from C-cell derived tumours
- Risk stratification in pre-malignant lesions
- Diagnosis of malignancy
- Prognosis
- Therapy selection

Follow-up
Repeated FNA
Total vs Partial thyroidectomy
Radioactive iodine
Targeted therapy

Somatic molecular alterations in thyroid carcinomas

Mutations: RAS family, BRAF, P53,…
Rearrangements: RET/PTC, PAX8-PPARγ,…
Amplifications and/or overexpression: MET, TGFβ,…
Deletions: Mitochondrial genes in Hürthle cell carcinomas,…
Epigenetic alterations: methylation, acetylation,…
siRNAs, miRNAs,…
……

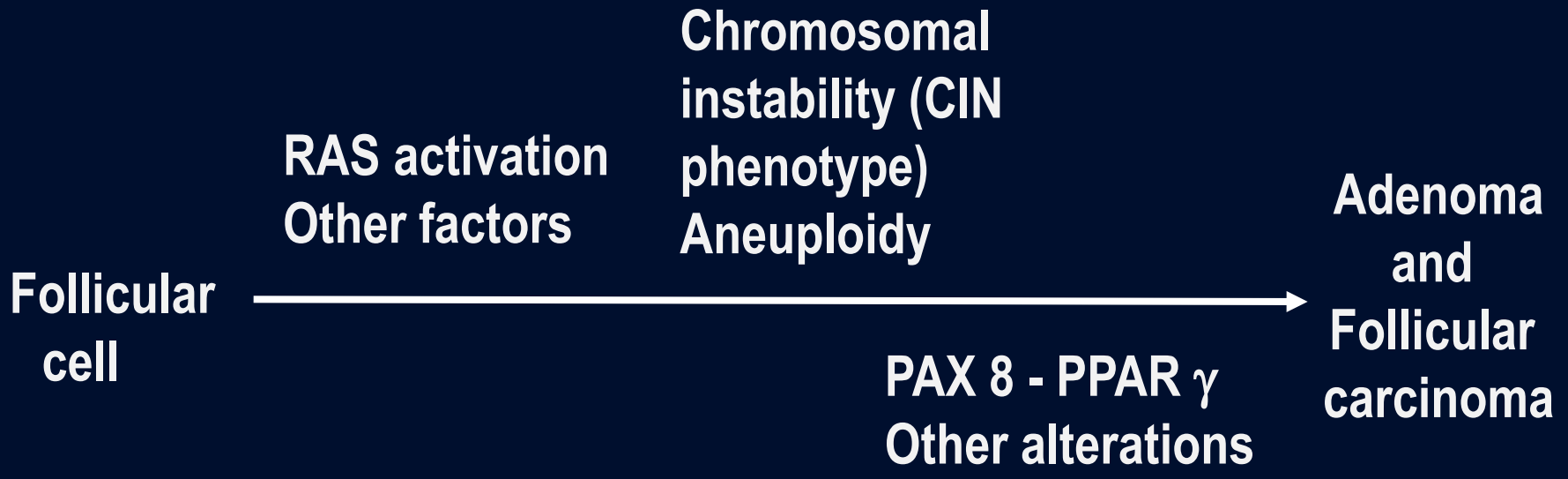
DIAGNOSIS

Major diagnostic problems in thyroid pathology

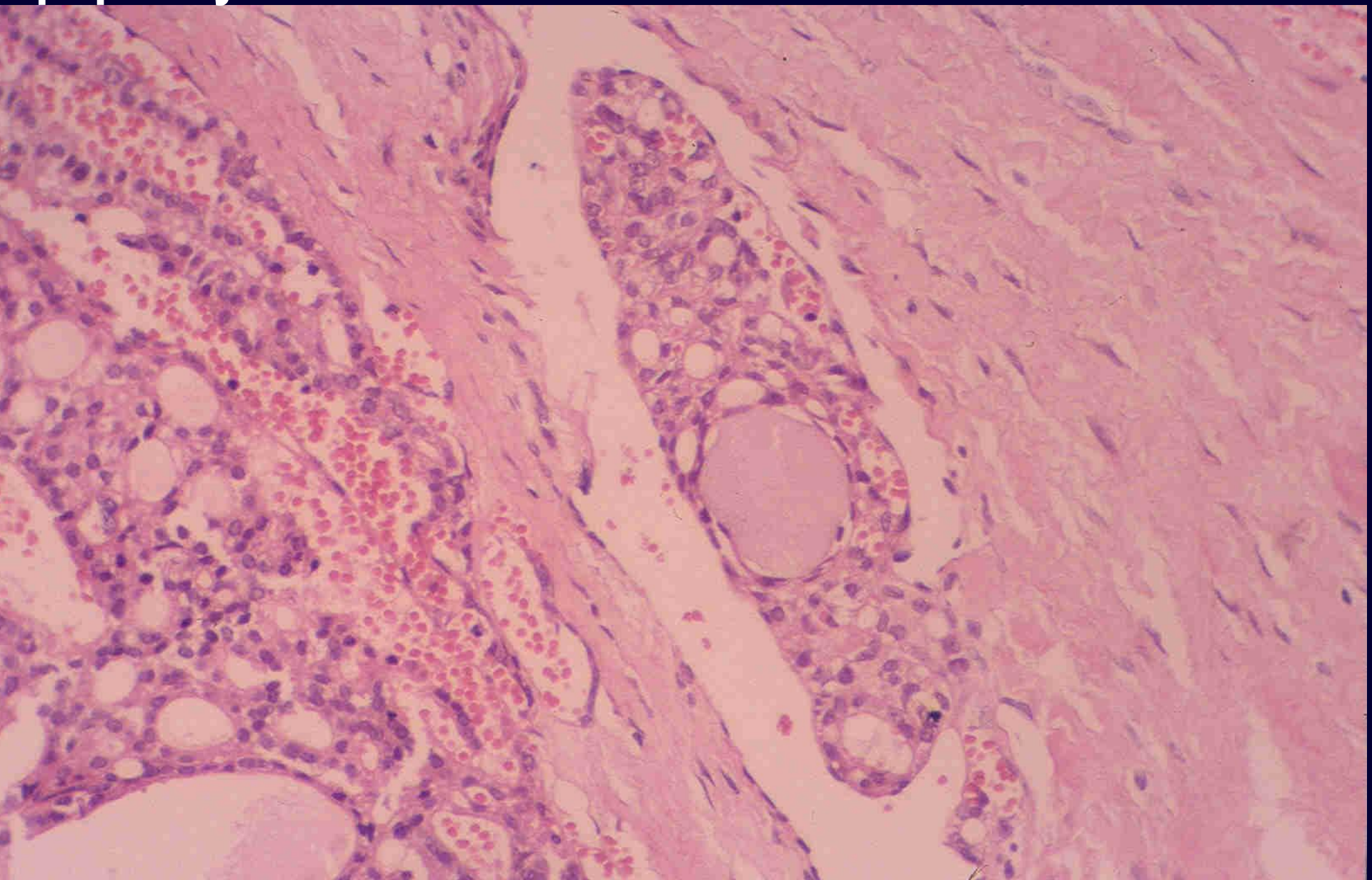
DD of poorly differentiated carcinoma
DD of minimally invasive follicular carcinoma & Encapsulated follicular variant of papillary carcinoma
DD of Hürthle cell tumours

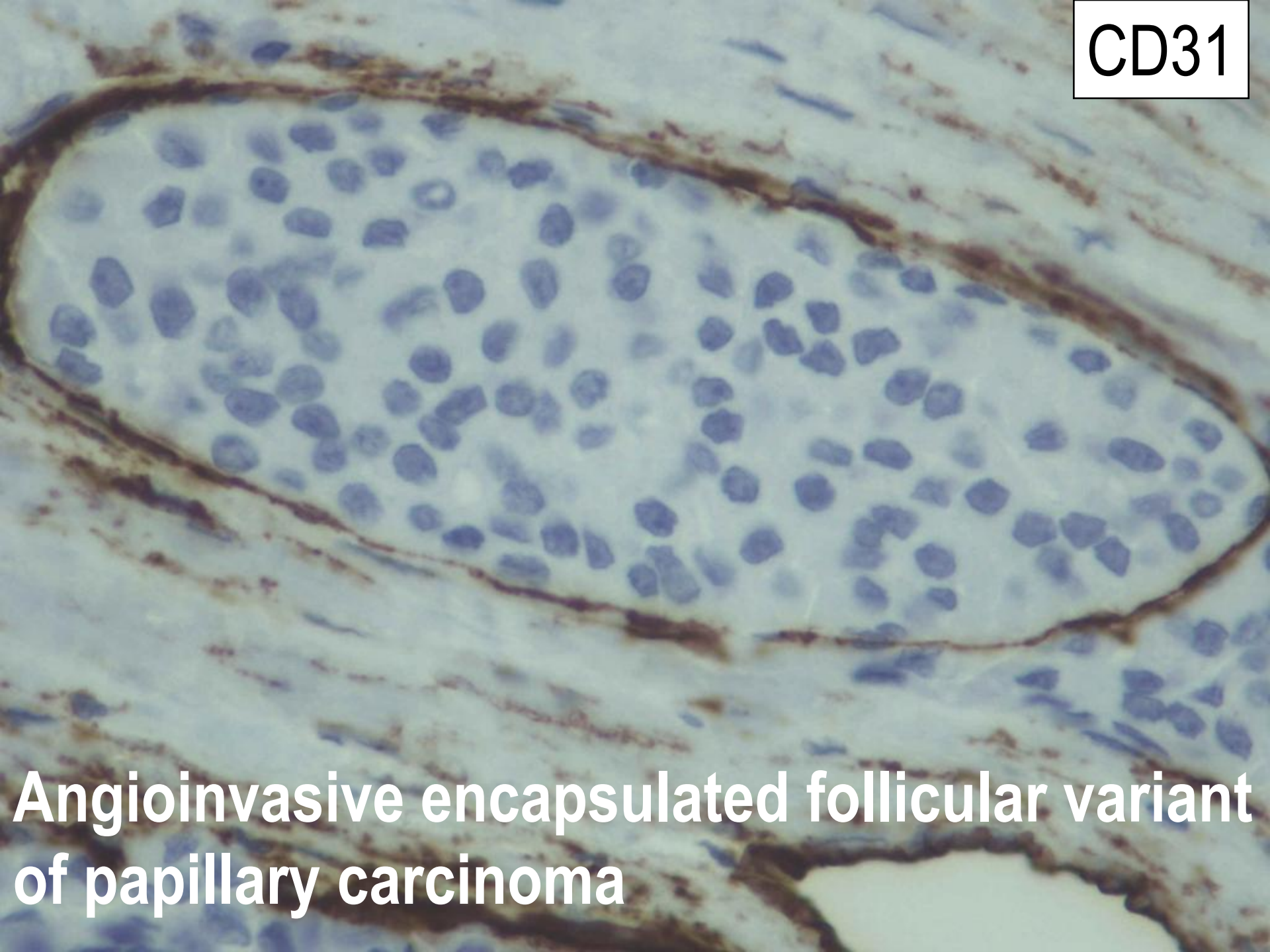
PROGNOSIS & THERAPY SELECTION

Multi-continental study on poorly differentiated thyroid carcinoma (PDCa)

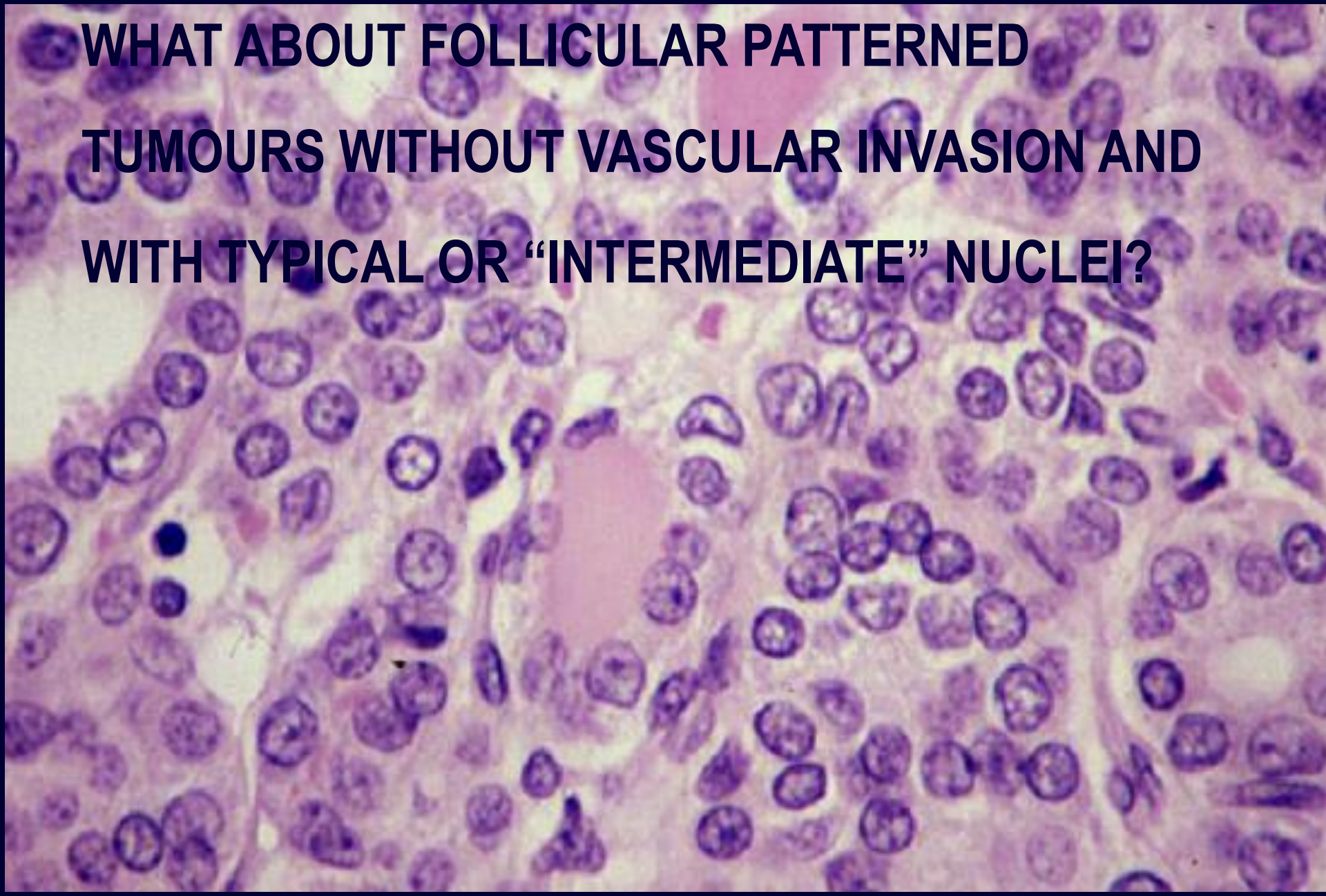

Chromosomal instability (CIN phenotype) Aneuploidy
RAS activation Other factors
Follicular cell
Adenoma and Follicular carcinoma
PAX 8 - PPAR γ Other alterations

Angioinvasive encapsulated follicular variant of papillary carcinoma





Angioinvasive encapsulated follicular variant of papillary carcinoma


WHAT ABOUT FOLLICULAR PATTERNED
TUMOURS WITHOUT VASCULAR INVASION AND
WITH TYPICAL OR “INTERMEDIATE” NUCLEI?

Classic dichotomic problem
Benign vs Malignant

Risk spectrum: "very low, low, intermediate,…"

GIST & Other tumours
Borderline malignancy & Uncertain malignant potential

……

240 cases (1978-2003) with nodal and/or distant metastases [Excluding medullary, poorly diff and undiff ca]

- Classical and several variants of PTC
- Poorly circumscribed and multinodular follicular variant of PTC
- Angio- and/or widely invasive follicular carcinoma

Consortium IPO-IPATIMUP, 2011 (unpublished results)

DIAGNOSIS

Major diagnostic problems in thyroid pathology

DD of poorly differentiated carcinoma
DD of minimally invasive follicular carcinoma & Encapsulated follicular variant of papillary carcinoma
DD of Hürthle cell tumours

PROGNOSIS & THERAPY SELECTION

First rule: Never rely upon a single or few genetic alterations