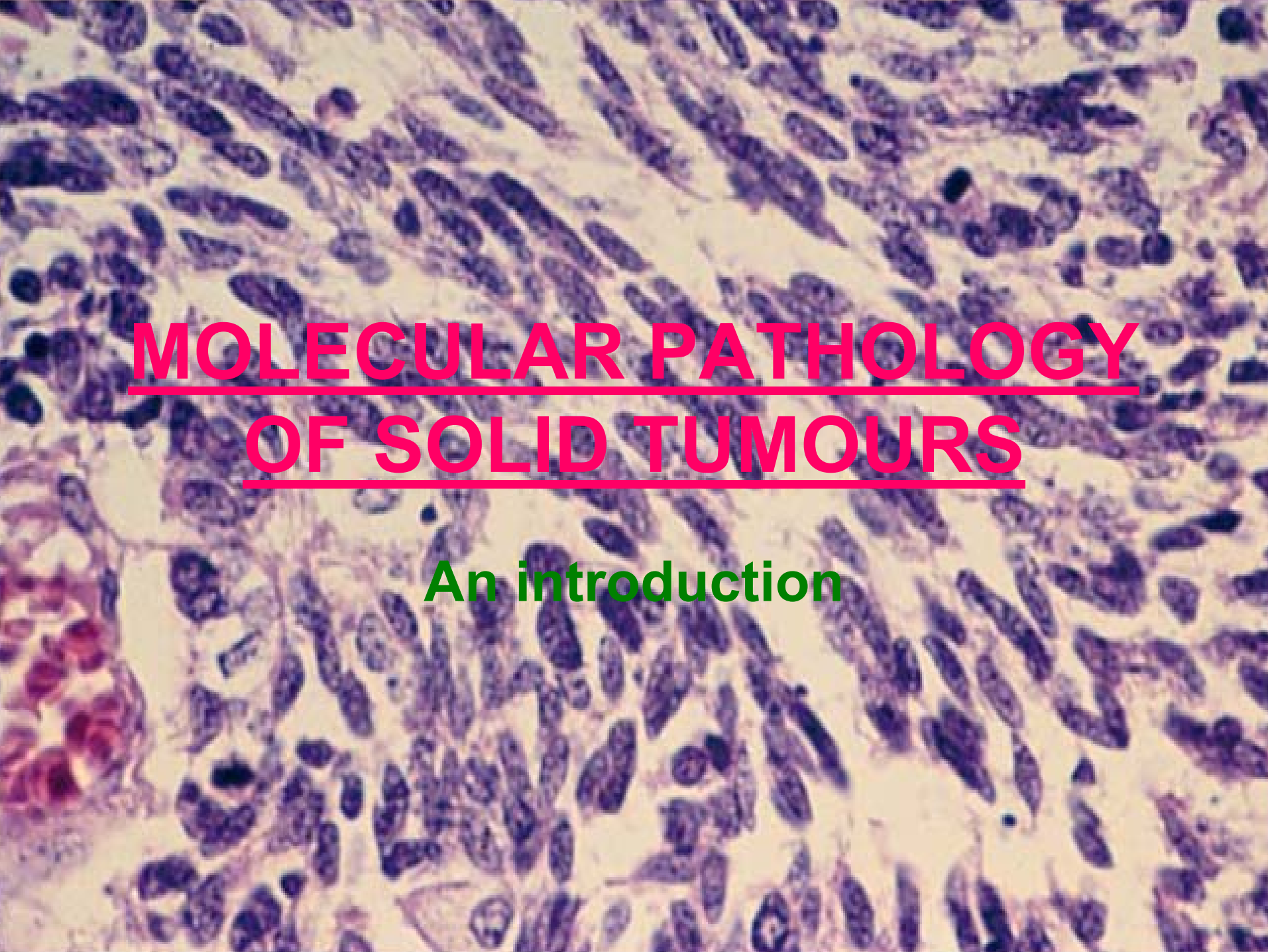
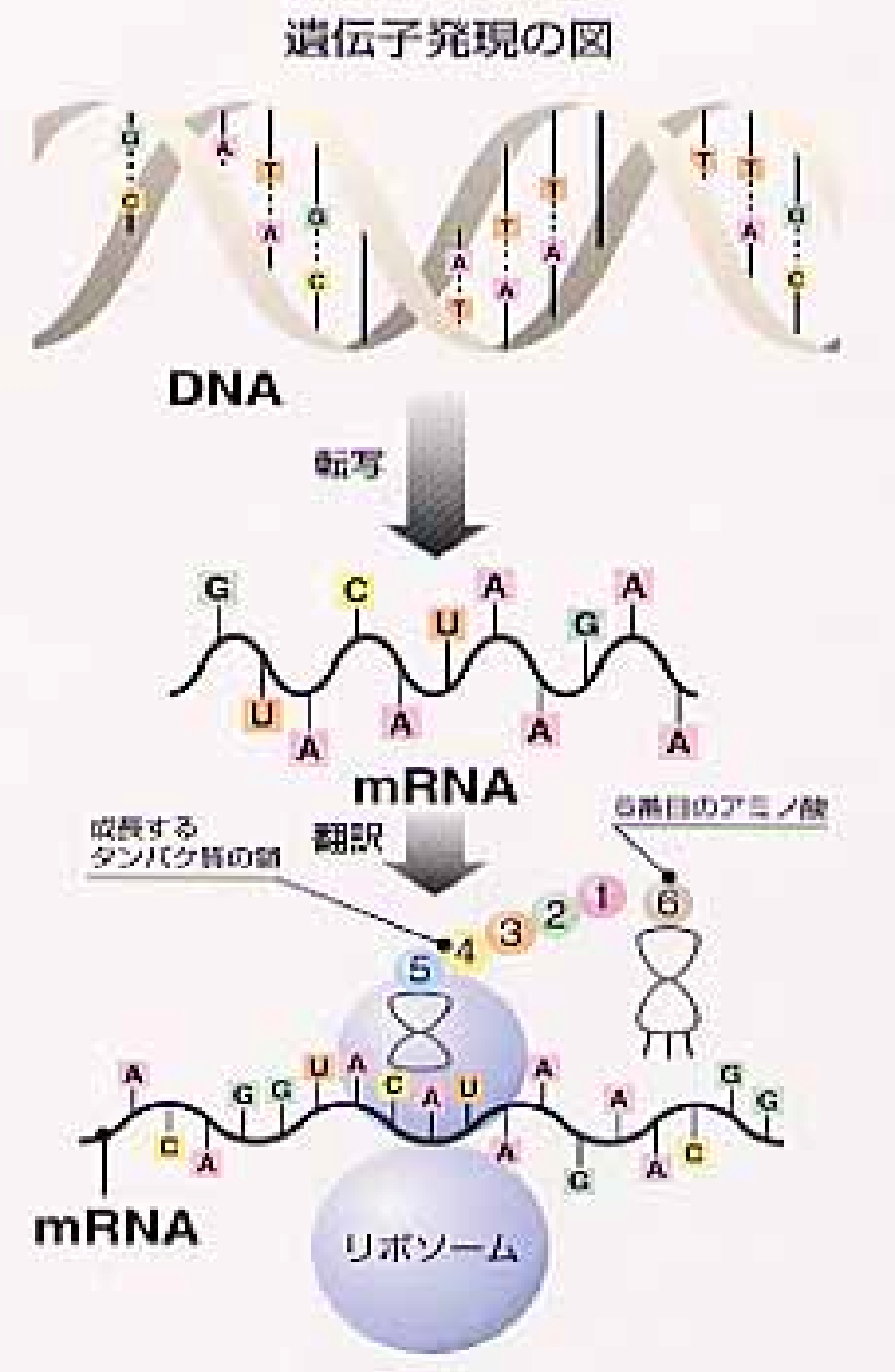


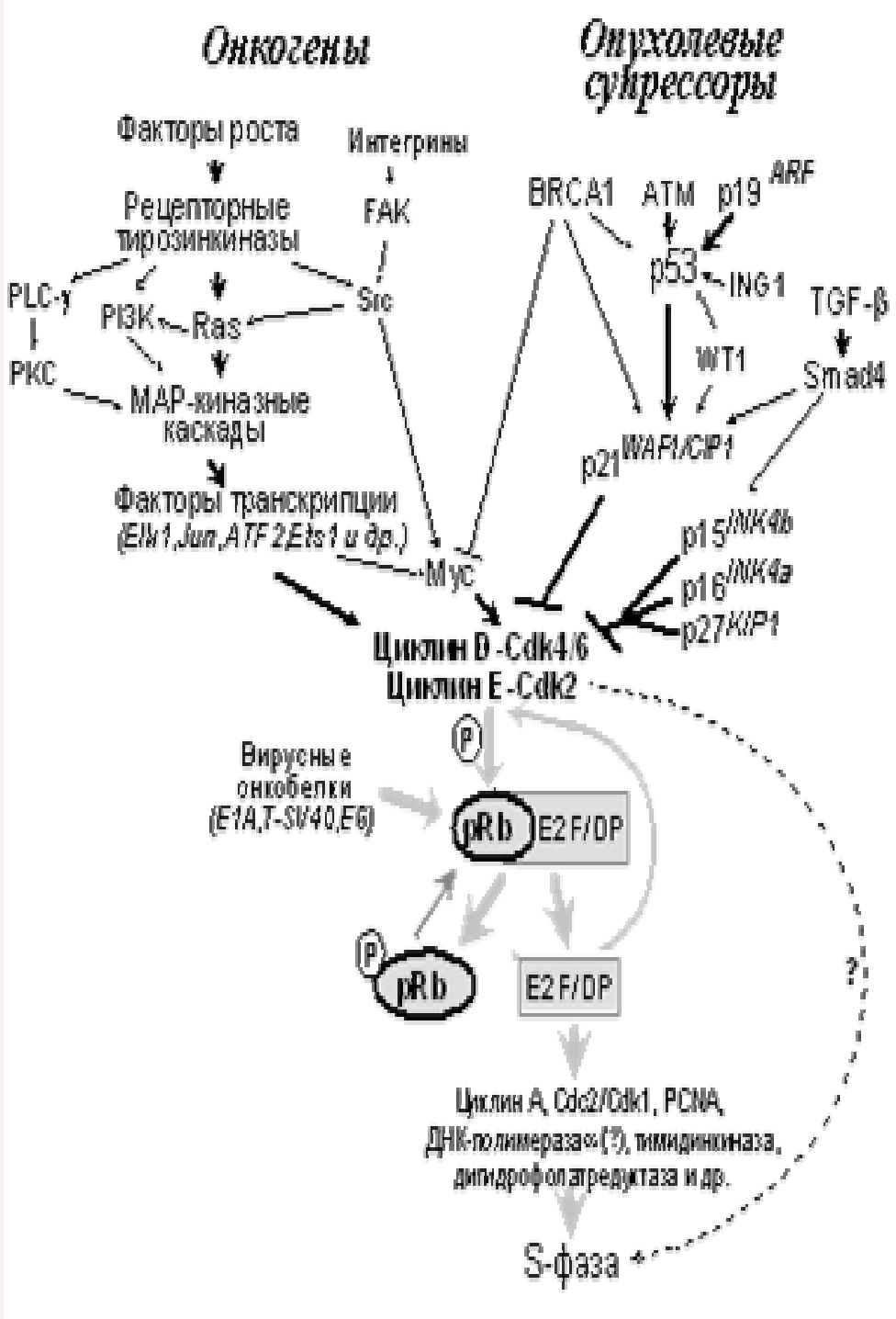
MOLECULAR PATHOLOGY OF SOLID TUMOURS

An introduction

GARDEN OF EDEN or GATE TO HELL FOR THE SURGICAL PATHOLOGIST ?

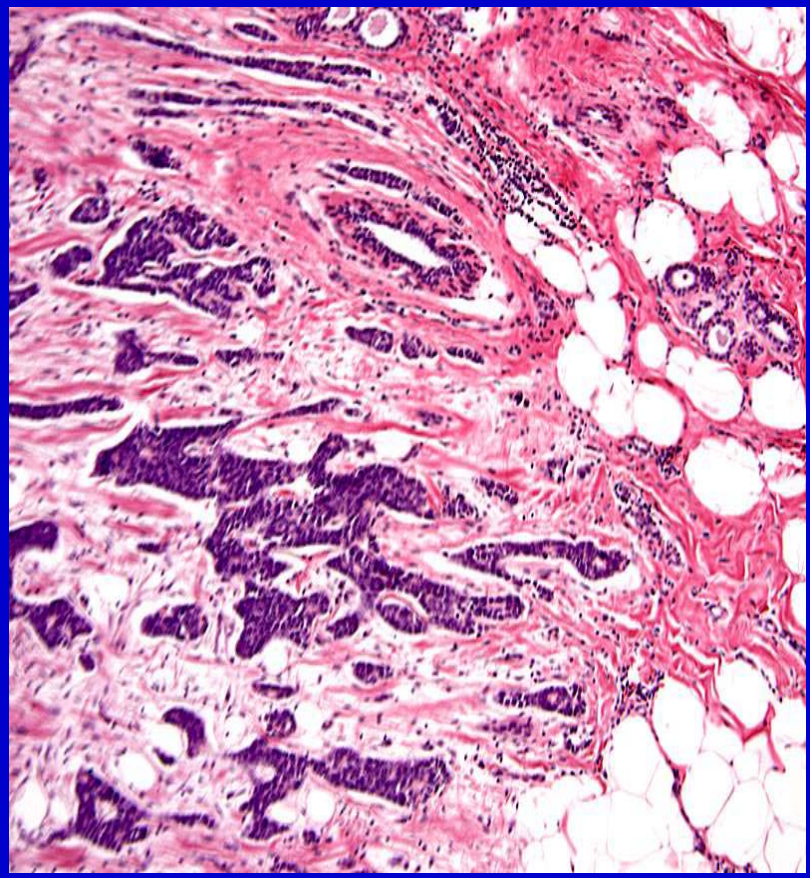
- Breast cancer
- Thyroid tumours
- Lung cancer
- Renal cell tumours


遺伝子発現の図
DNA
転写
mRNA
翻訳
成長するタンパク質の鎖
6番目のアミノ酸
mRNA
リボソーム


Онкогены
Опухолевые супрессоры
Факторы роста
Интегрины
Рецепторные тирозинкиназы
FAK
BRCA1
ATM
p19 ARF
p53
ING1
PLC-γ
PI3K
Ras
Src
TGF-β
WT1
Smad4
PKC
MAP-киназные каскады
p21 WAF1/CIP1
Факторы транскрипции (Elk1, Jun, ATF2, Ets1 и др.)
Myc
p15 INK4b
p16 INK4a
p27 KIP1
Циклин D-Cdk4/6
Циклин E-Cdk2
Вирусные онкобелки (E1A, T-SV40, E6)
pRb
E2F/DP
Циклин A, Cdc2/Cdk1, PCNA, ДНК-полимераза α (?), тимидинкиназа, дигидрофолатредуктаза и др.
S-фаза

Breast Cancer

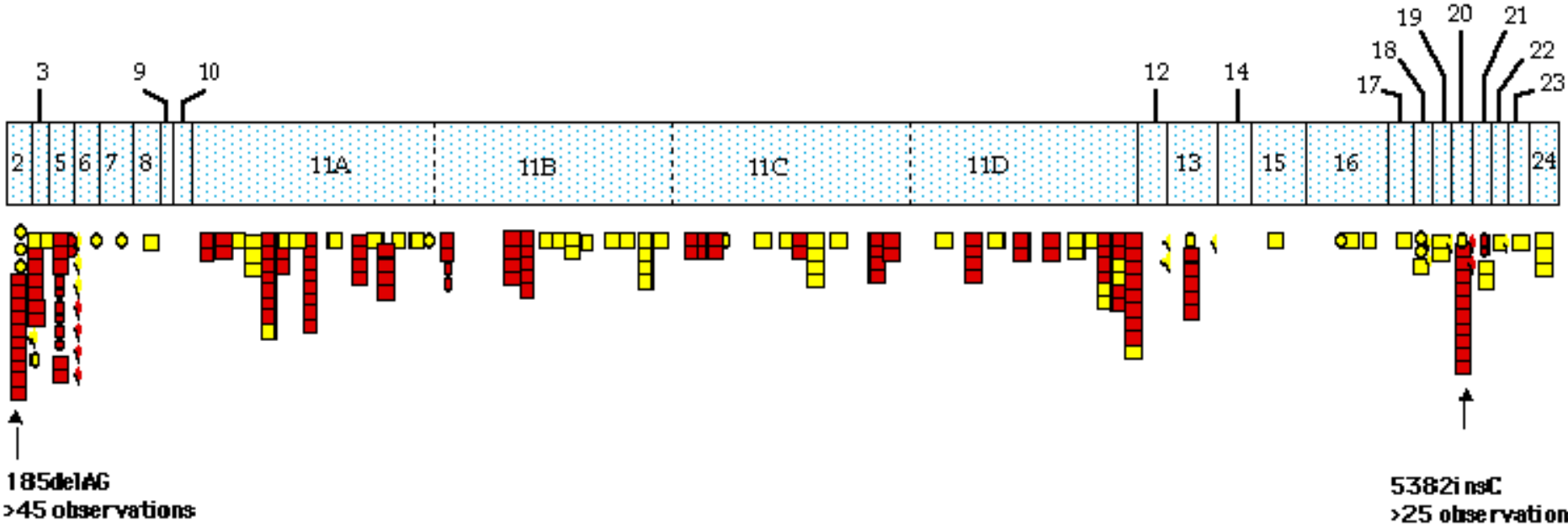
- **Hereditary**
- **Sporadic**



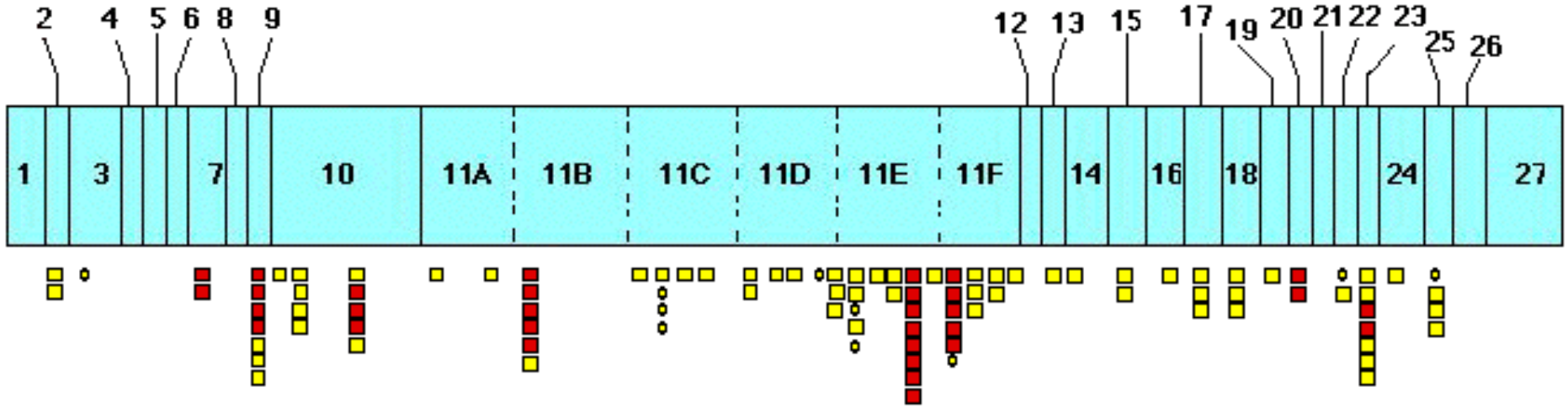
Hereditary

- BRCA-1 5%
- BRCA-2 3%
- P53 0.1%
- PTEN/MMAC mutation 0.1 %

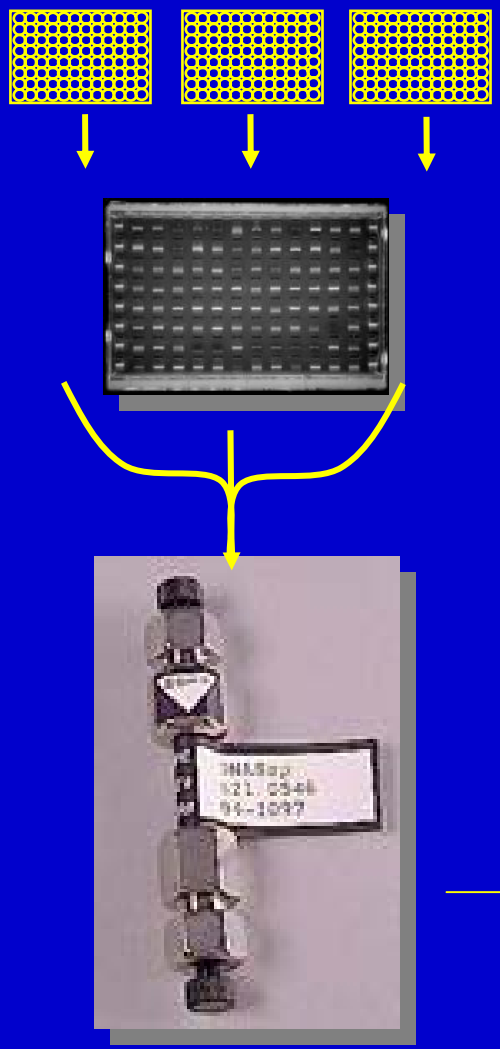
BRCA1 Condensed Mutation Database



BRCA2 Condensed Mutation Database



Strategy for « large genes »

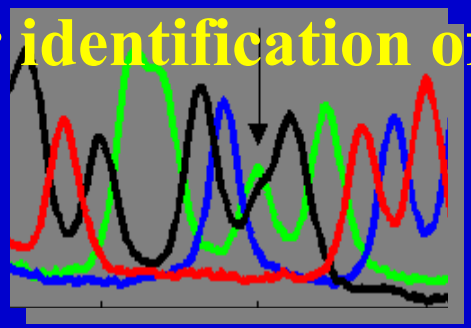


PCR amplification of 40 fragments
Check PCR on agarose

Mix PCR products to form heteroduplexes

Analysis with DHPLC

Sequencing of abnormal fragments for identification of mutations



BRCA1-2 mutation analysis

- Screening of 40 PCR fragments
- denaturing HPLC
- Deletion analysis
- Sequence analysis

BRCA1-2 mutation analysis

- Expensive, time consuming
  - No mass screening for BRCA1-2 mutations
    - Few percent positive
  - Selection of families

Familial breast carcinoma

- Familial ≠ hereditary
  - cum risk 80y: 8-9% voor vrouw
- In 5% genetic factor
  - monogenetic problem: BRCA1-2, ...
- 10-15 % familial clustering
- multifactorial problem
- Remaining: sporadic

Indications for mutation analysis

- Good selection of families
  - >= 3 patients
  - families with breast en ovary cancer
  - families with male breast cancer (BRCA2)
  - Breast cancer < 30j (single patient)
  - minimally1 patient alive
    - Preferentially two as frequent incidence of sporadic cases

Risk breast and ovary cancer

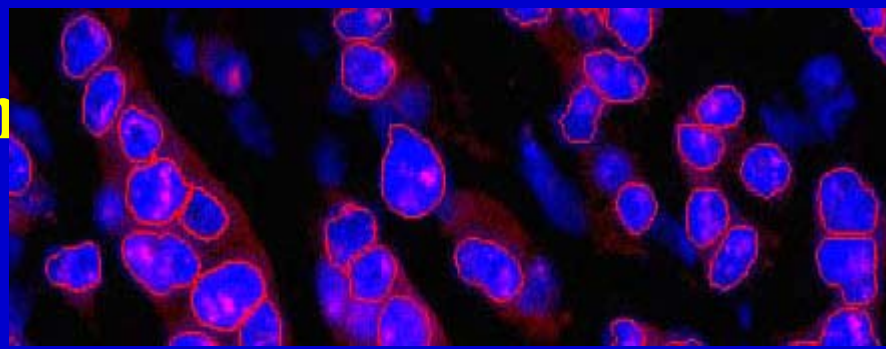
Cumulative Incidence		BRCA1	BRCA2	Population Risk
Breastcarc	50y 70y	0.49 0.71	0.28 0.84	0.012 0.052
Bilateraal BC	70y	0.64	0.52	-
Ovariumcarc.	50y 70y	0.11 0.29	0.03 0.27	0.002 0.009
Man BC	70y	-	0.06	-

Preventive strategy for males with BRCA1-2 mutation

- **1X year preventive investigation for prostate carcinoma: start 50 years.**
  - **PSA dosage**

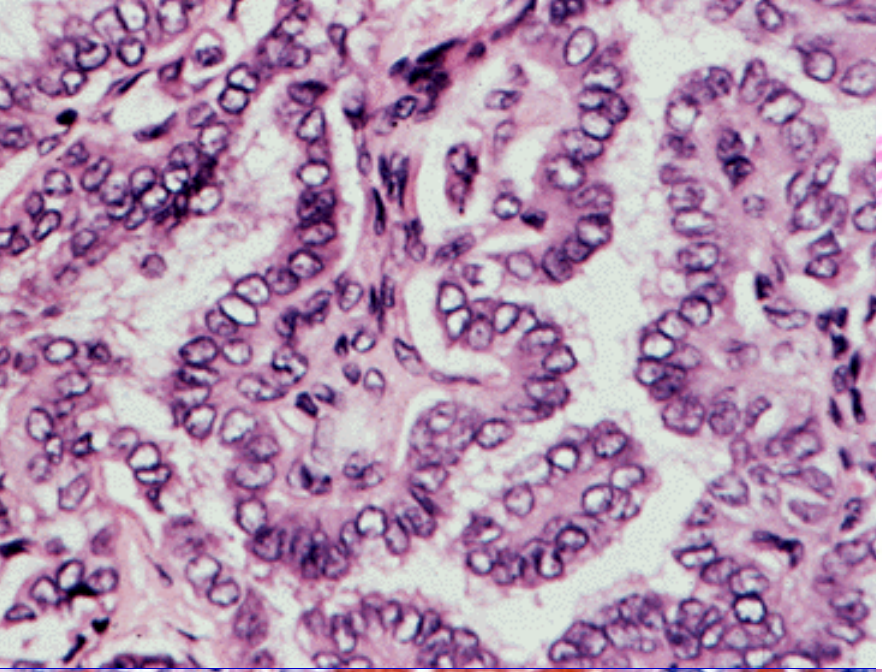
Acquired

- Erb-b2 amplification
- Myc-amplification
- CCND 1 amplification
- AIB 1 amplification
- P53 mutation
- Rb-1 mutation
- P16 methylation

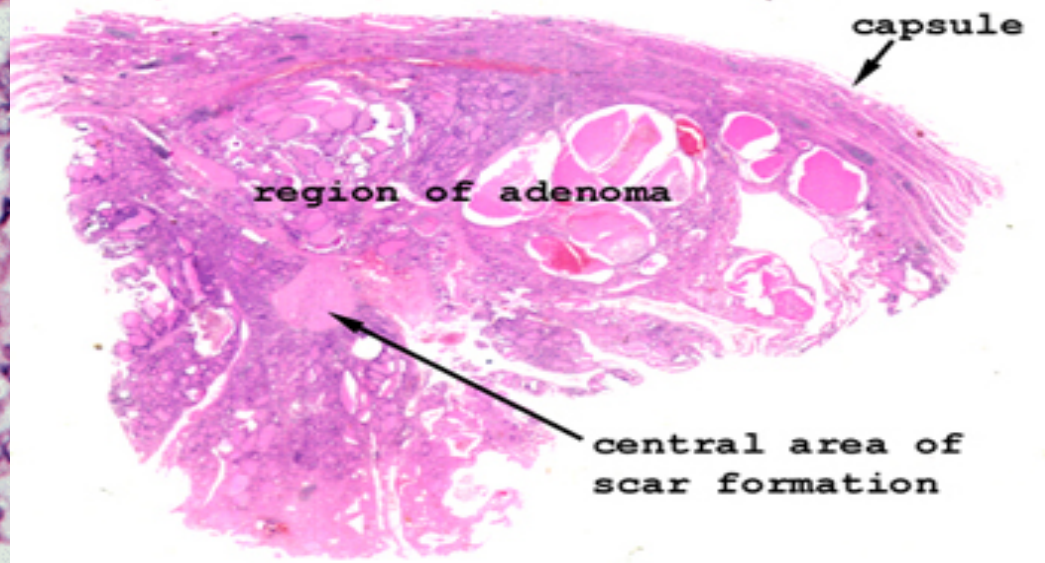


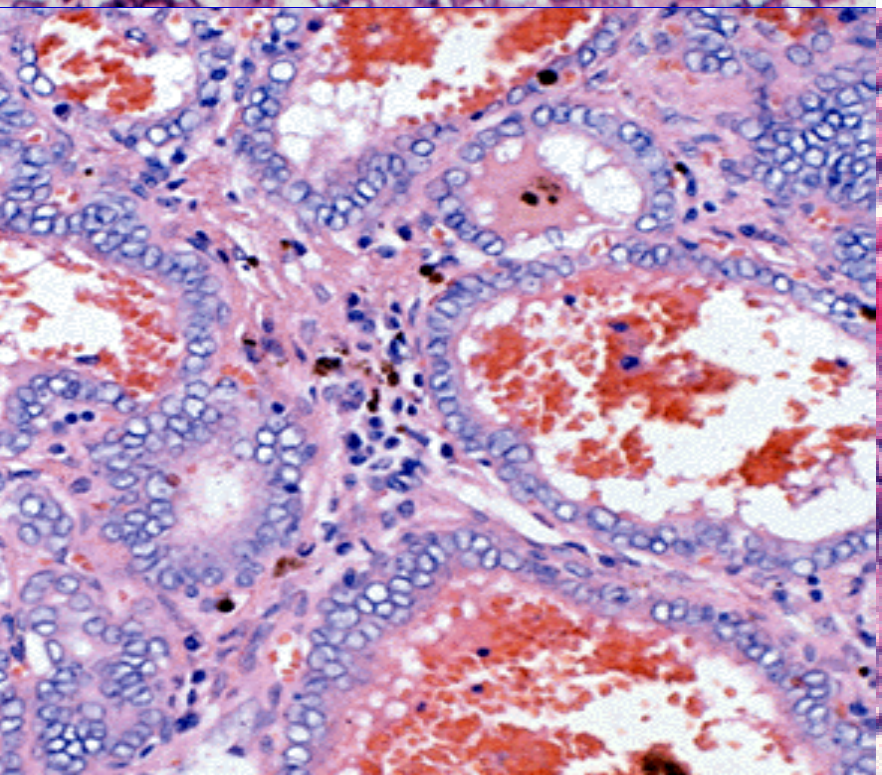
Prognosis-Therapy

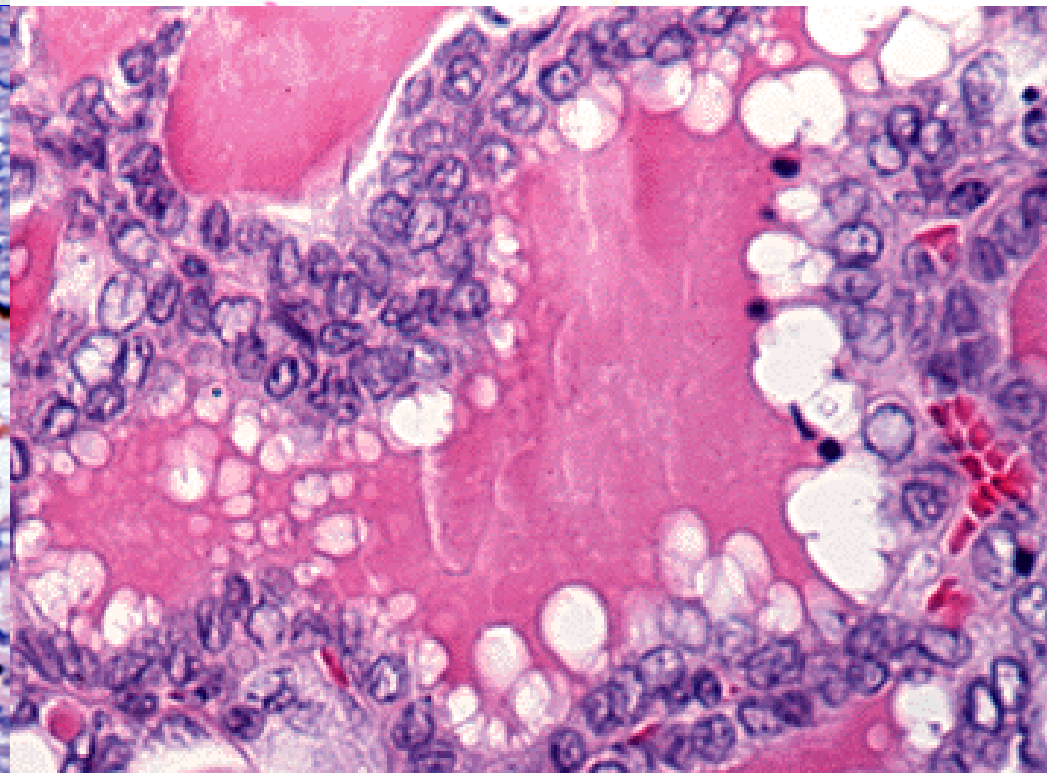
- **Hormone receptors**
- **Growth factor receptors (EGF-R)**
- **Erb-B2**



This is actually only a small wedge out of the adenoma.

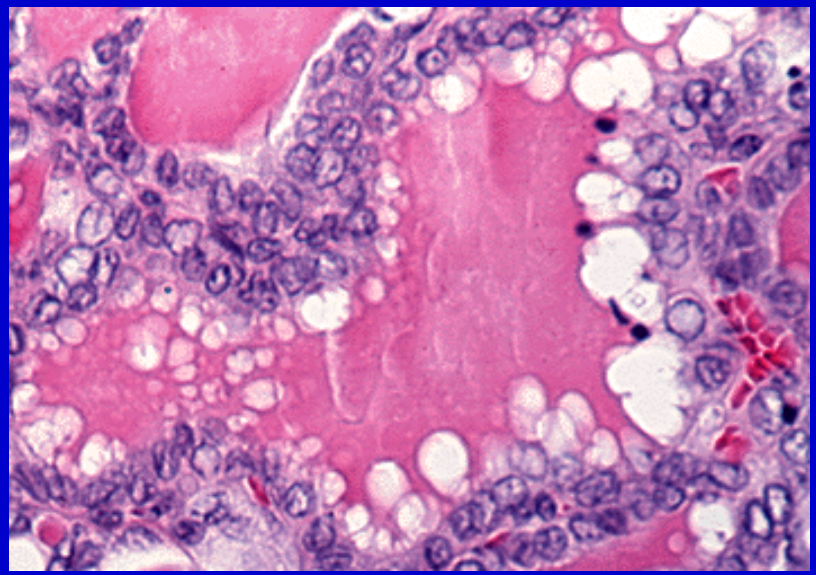






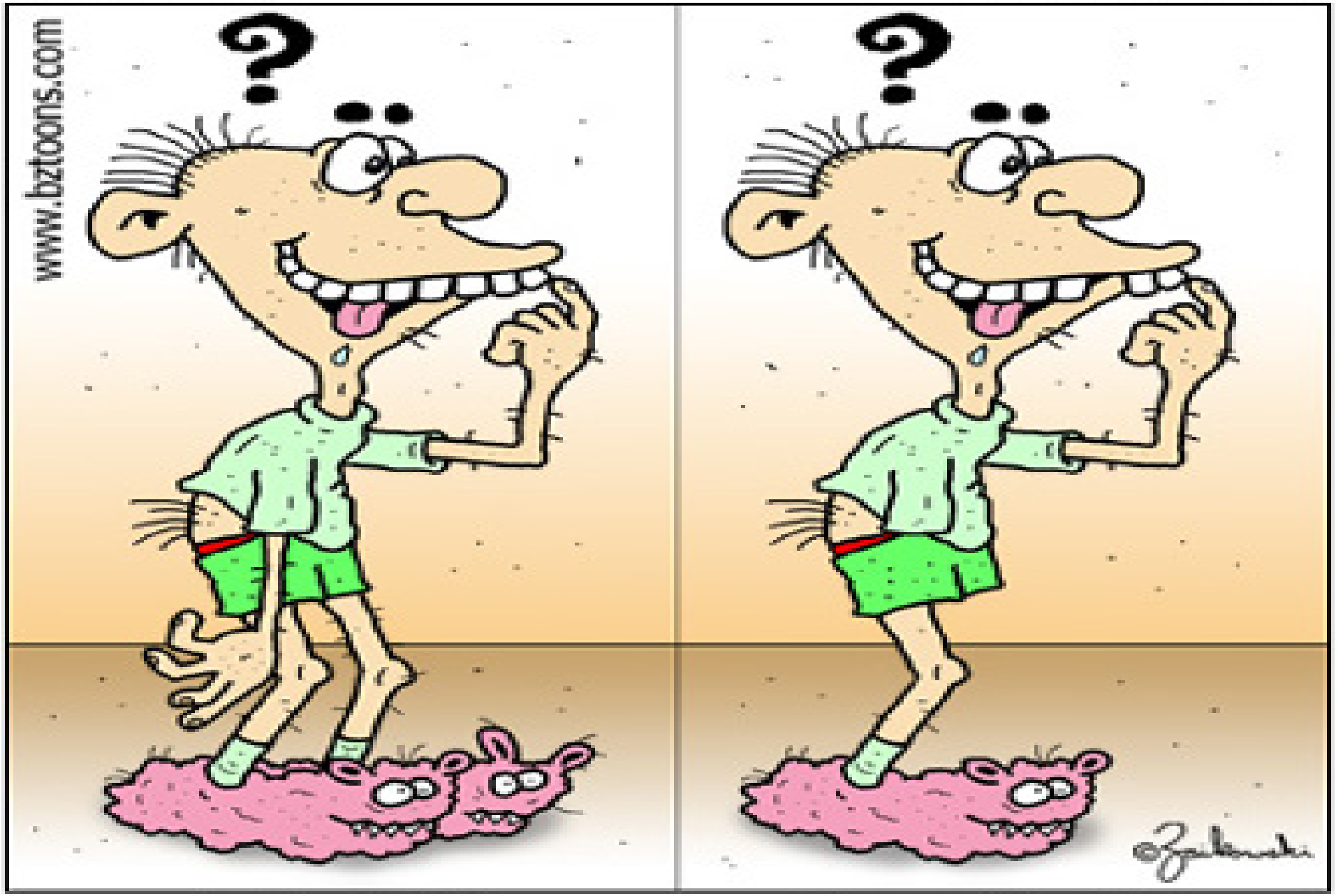
THYROID TUMOURS

- **Follicular adenoma**
- **Carcinoma**



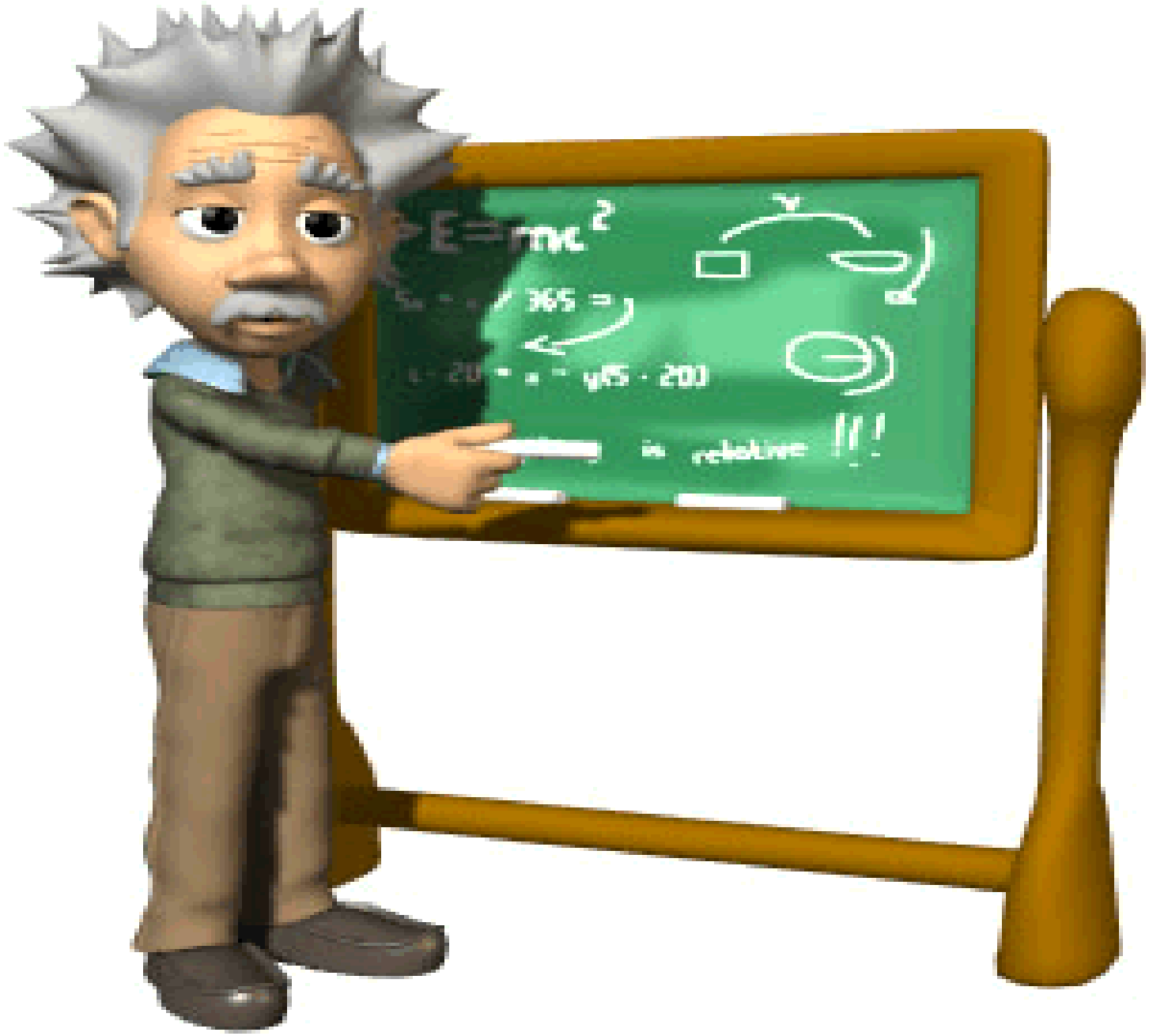
B.Z. Toons

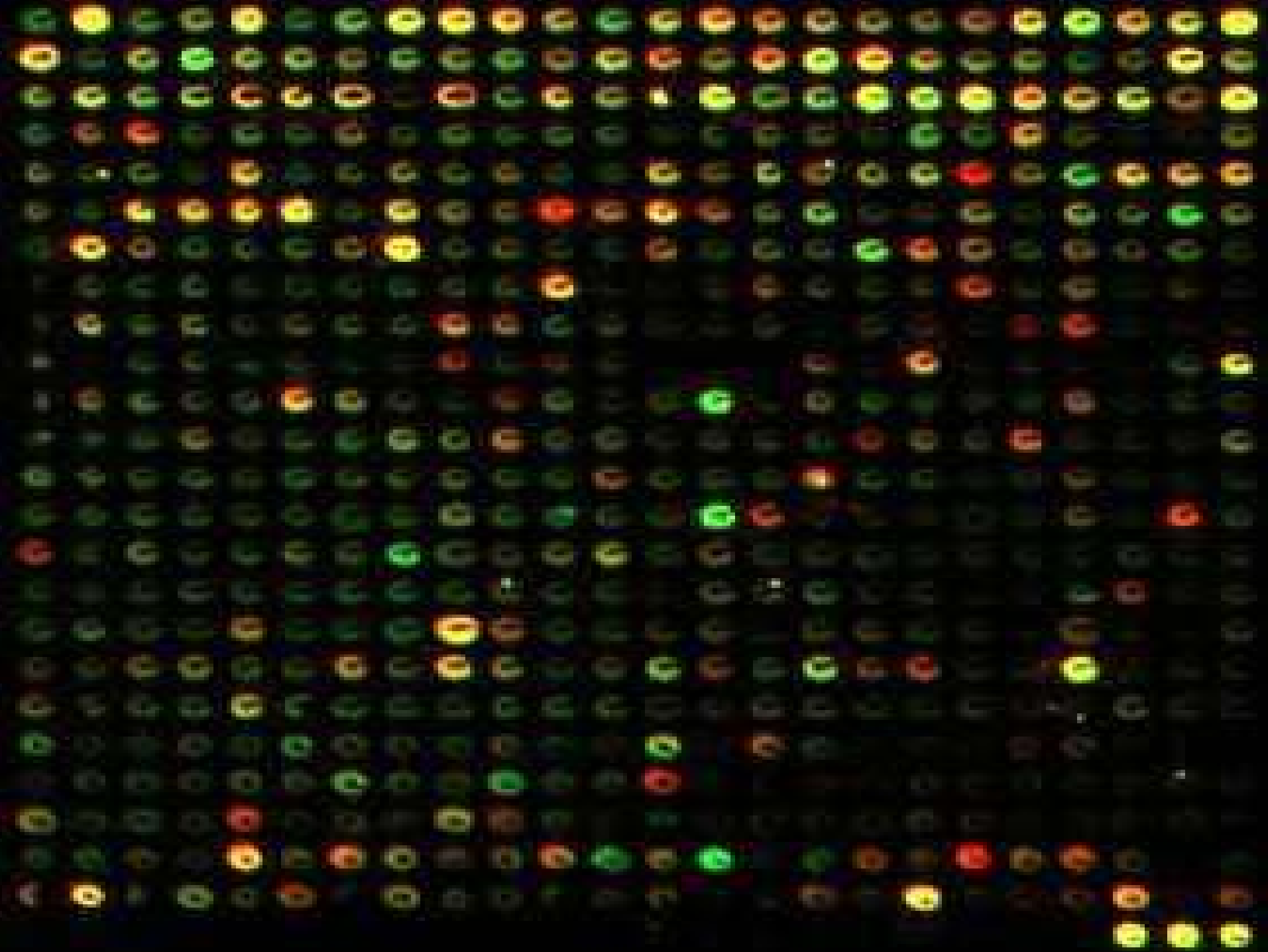
by Brian Zaikowski

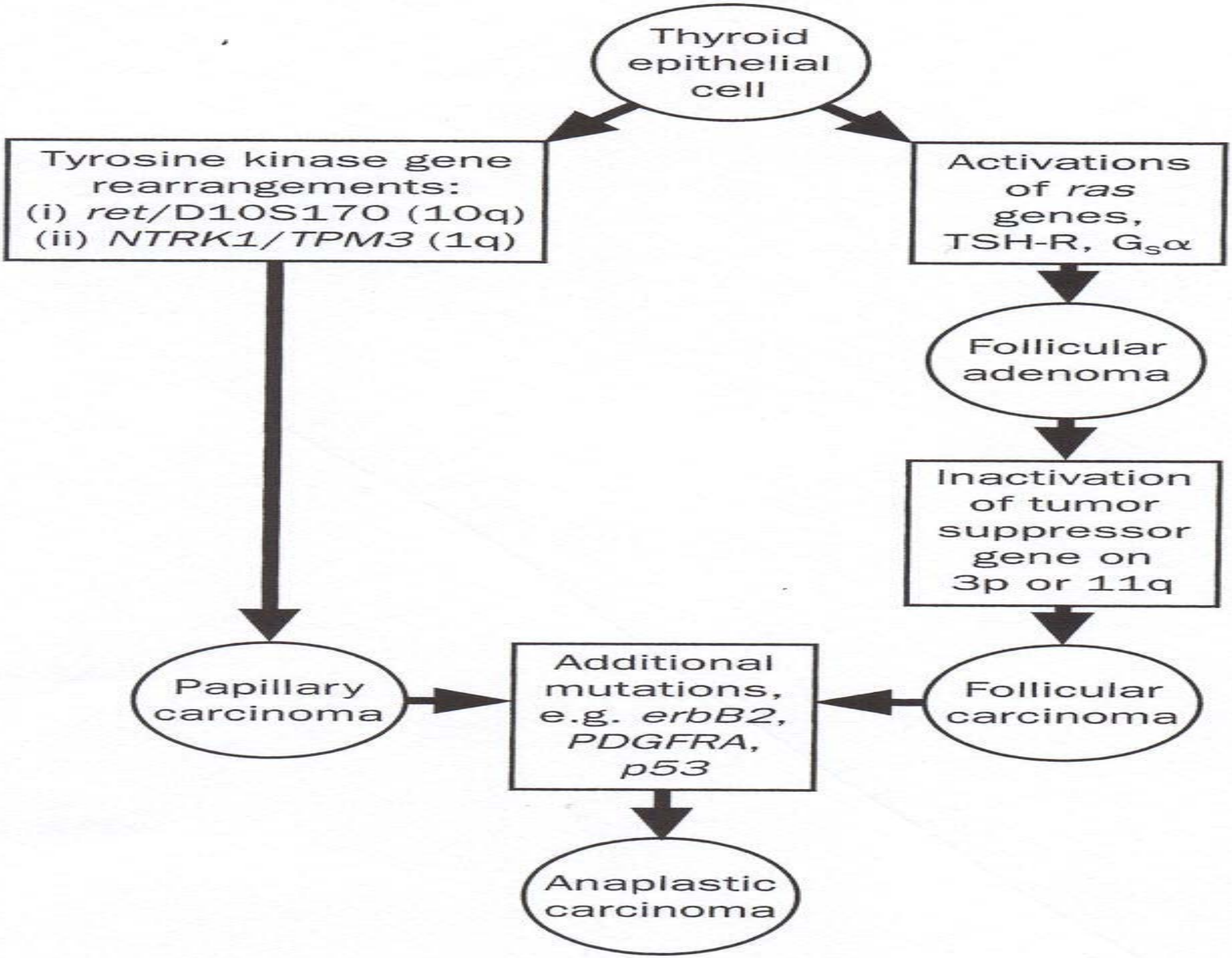


Complete idiot

Incomplete idiot


$E=mc^2$
365 =
is relative !!!



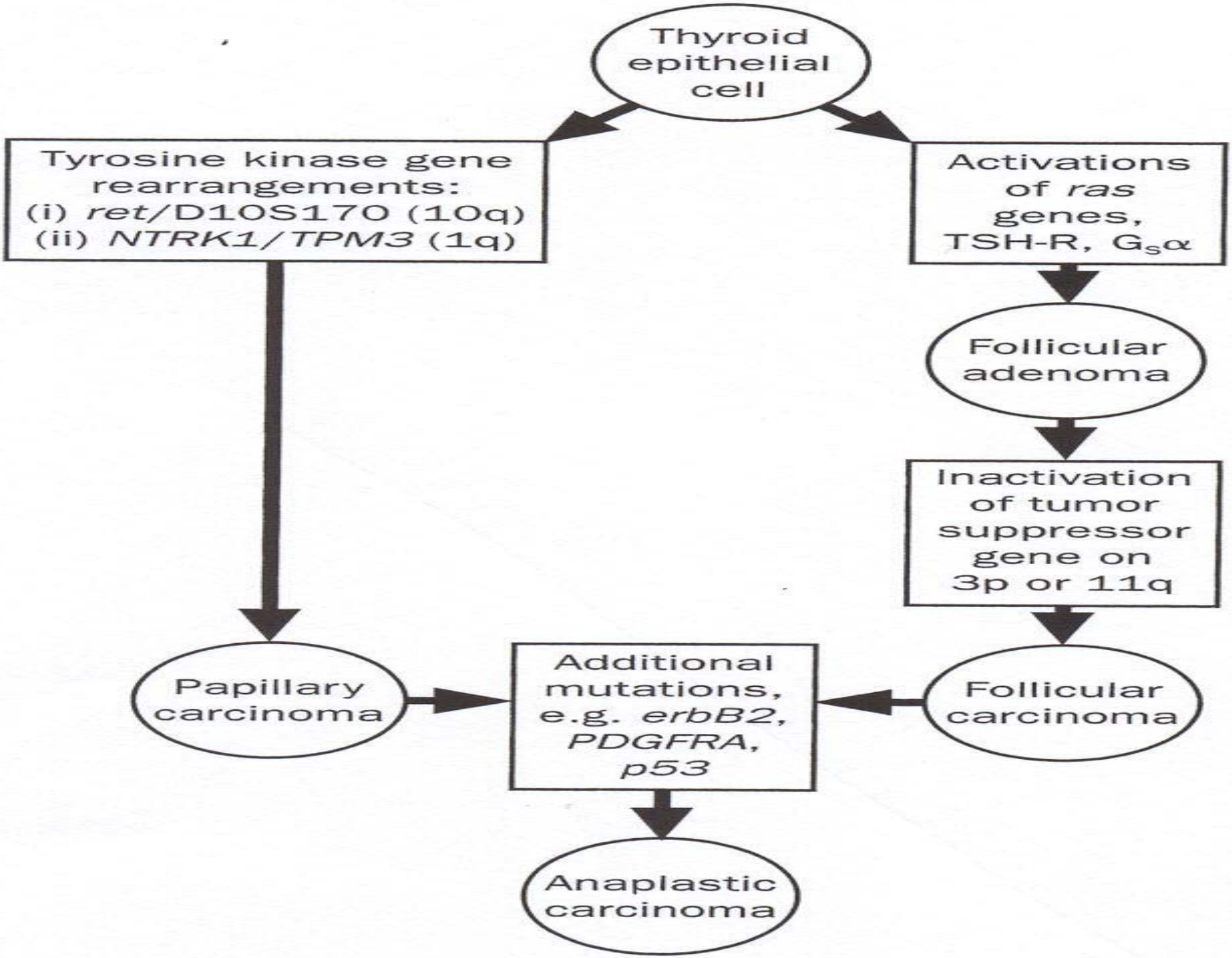

Thyroid epithelial cell
Tyrosine kinase gene rearrangements:
(i) ret/D10S170 (10q)
(ii) NTRK1/TPM3 (1q)
Activations of ras genes, TSH-R, $G_s\alpha$
Follicular adenoma
Inactivation of tumor suppressor gene on 3p or 11q
Papillary carcinoma
Additional mutations, e.g. erbB2, PDGFRA, p53
Follicular carcinoma
Anaplastic carcinoma

Thyroid cancer

- Papillary carcinoma 70%
- Follicular carcinoma 15%
- Anaplastic carcinoma 10%
- Medullary carcinoma 5%

Table 17.2 Thyroid cancers and associated genetic abnormalities

Thyroid neoplasm	Oncogene (chromosomal location)	
	Dominant	**Recessive**
Papillary	*ret/PTC* (10q11–q12) *NTRK1* (1q32–q41)	
Follicular (adenoma/carcinoma)	TSH-R (22q11–q13) $G_s\alpha$ (20q) Ha-*ras* (11p15.5) N-*ras* (1p13) Ki-*ras* (12p12.1)	*FTC* (3p) 11q
Anaplastic	Ki-*ras* (12p12.1) *PDGFRA* (4q12) *erbB2* (17q21–q22) *EGFR* (7p13.22)	
Medullary	*ret/MEN2A, MEN2B* *MTC* (10q11.2) Ha-*ras* (11p15.5) c-*myc* (8q24.12) N-*myc* (2p24.1)	1p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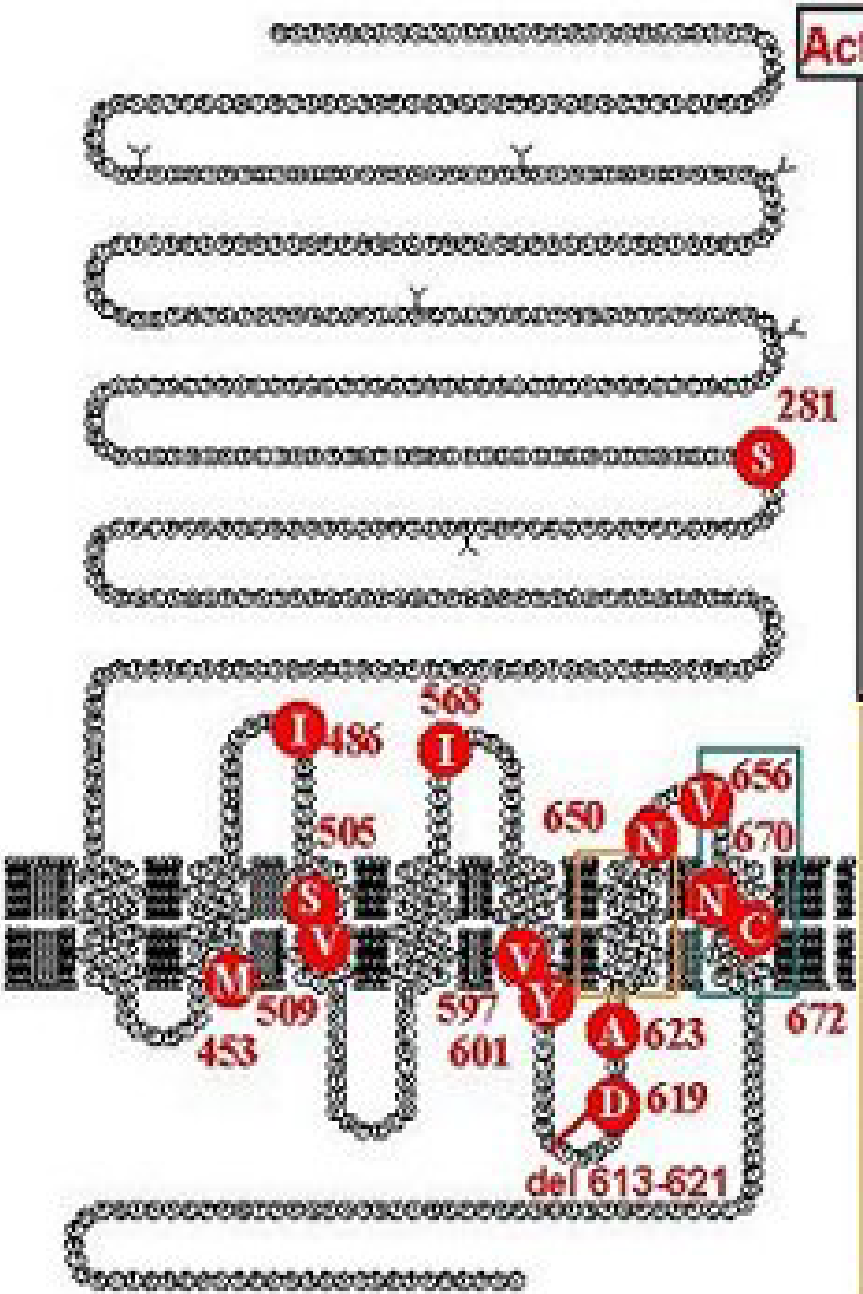
Activations of ras genes, TSH-R, $G_s\alpha$
Follicular adenoma
Inactivation of tumor suppressor gene on 3p or 11q
Papillary carcinoma
Additional mutations, e.g. erbB2, PDGFRA, p53
Follicular carcinoma
Anaplastic carcinoma

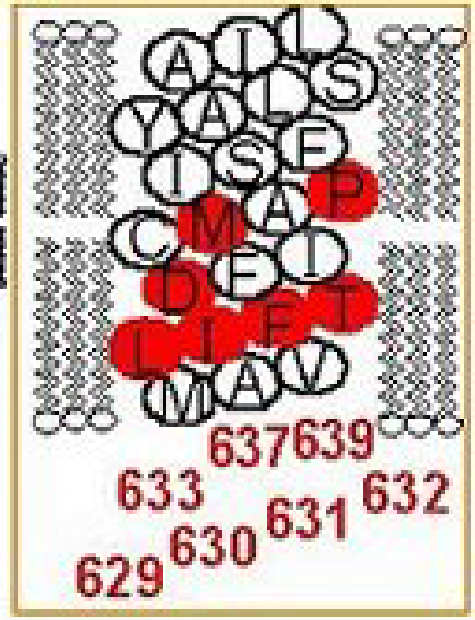
"HOT NODULE"

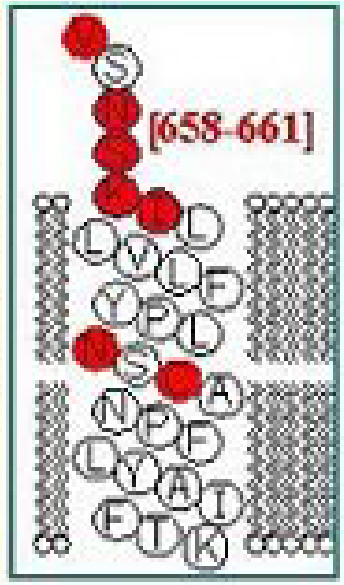
- **TSH-receptor activating mutations** **25-65%**
- **G-coupled receptor** **30%**

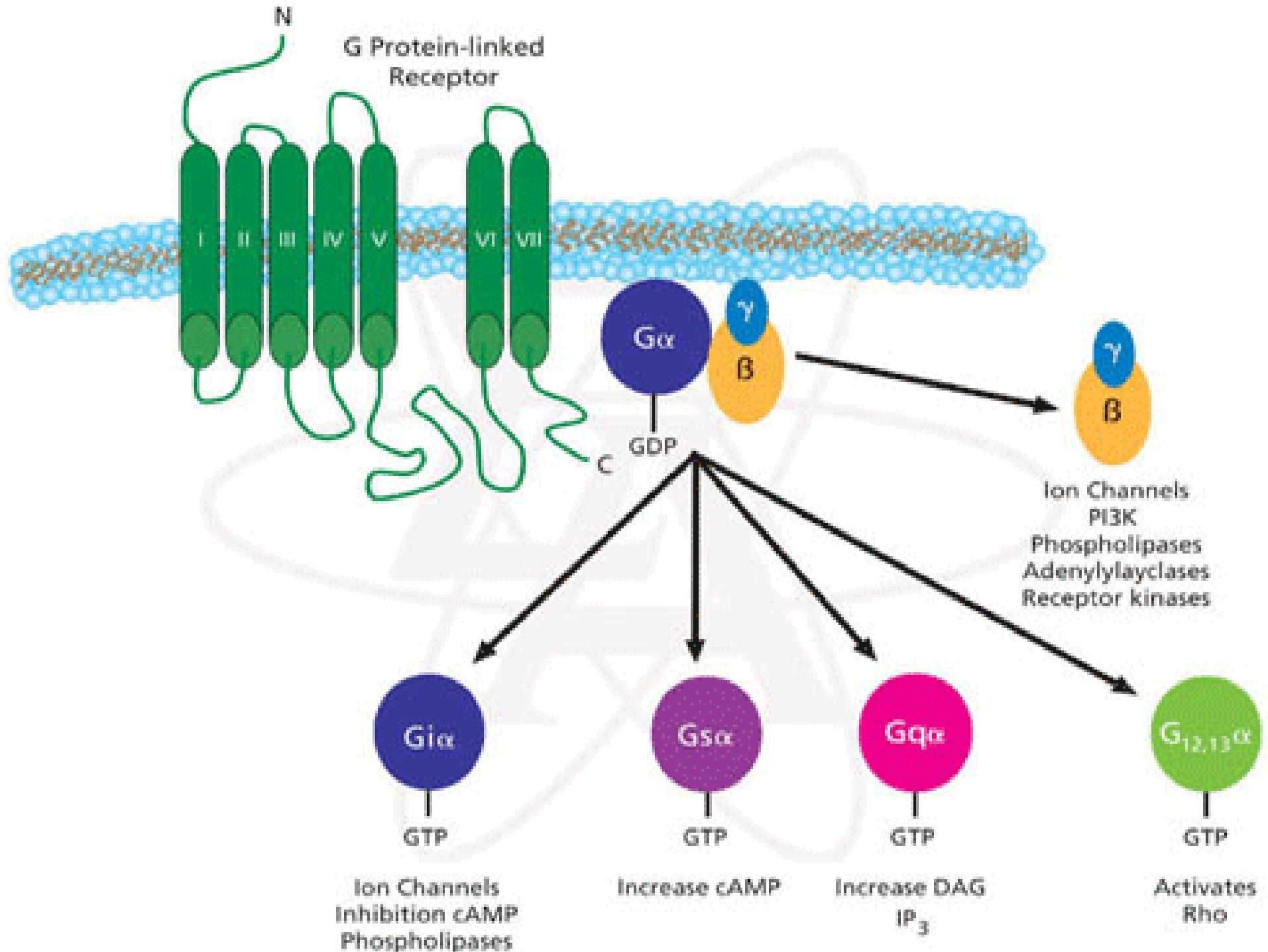
Activating mutations in TSH receptor

Ser	281	Thr/Asn/Ile	Ile	630	Leu
Met	453	Thr	Phe	631	Leu/Cys
Ile	486	Phe/Met	Thr	632	Ile / Ala
Ser	505	Arg/Asn	Asp	633	Glu/Tyr/His/Ala
Val	509	Ala	Met	637	Arg
Ile	568	Thr	Pro	639	Ser
Val	597	Leu	Asn	650	Tyr
Tyr	601	Asn	Val	656	Phe
Del	613-621		Del	658-661	
(YNPGDKDTK)			(NSKI)		
Asp	619	Gly	Asn	670	Ser
Ala	623	Ile/Val/Ser	Cys	672	Tyr
Leu	629	Phe /Pro			



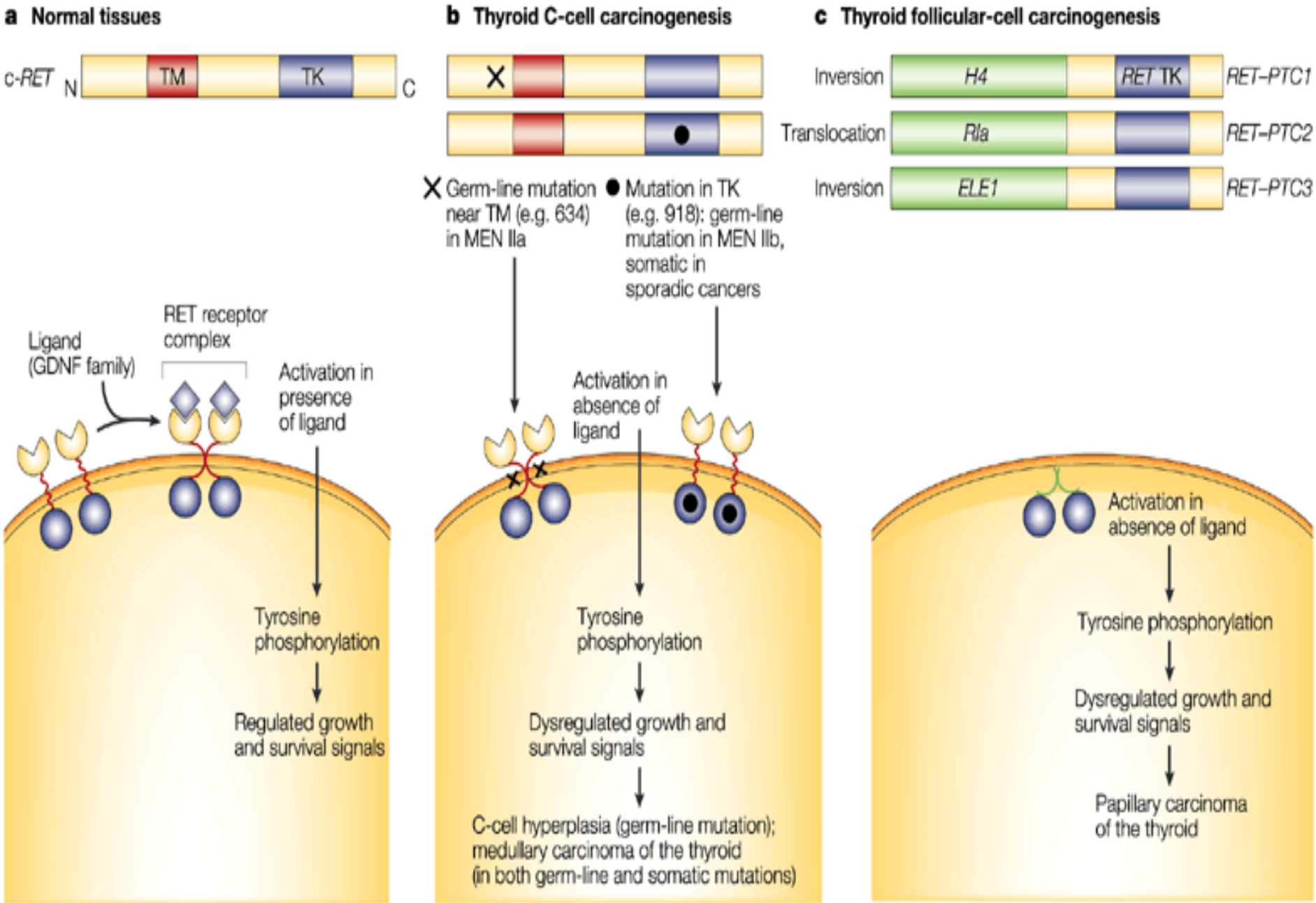





N
G Protein-linked
Receptor
I
II
III
IV
V
VI
VII
C
Gα
γ
β
GDP
γ
β
Ion Channels
PI3K
Phospholipases
Adenylylayclases
Receptor kinases
Giα
GTP
Ion Channels
Inhibition cAMP
Phospholipases
Gsα
GTP
Increase cAMP
Gqα
GTP
Increase DAG
IP3
G12,13α
GTP
Activates
Rho

Papillary carcinoma

- PTC gene
- Fusion gene ret proto-oncogene/ D10S170
- NTRK gene
- Increased expression c-erb-B2, c-fos, c-myc, ras

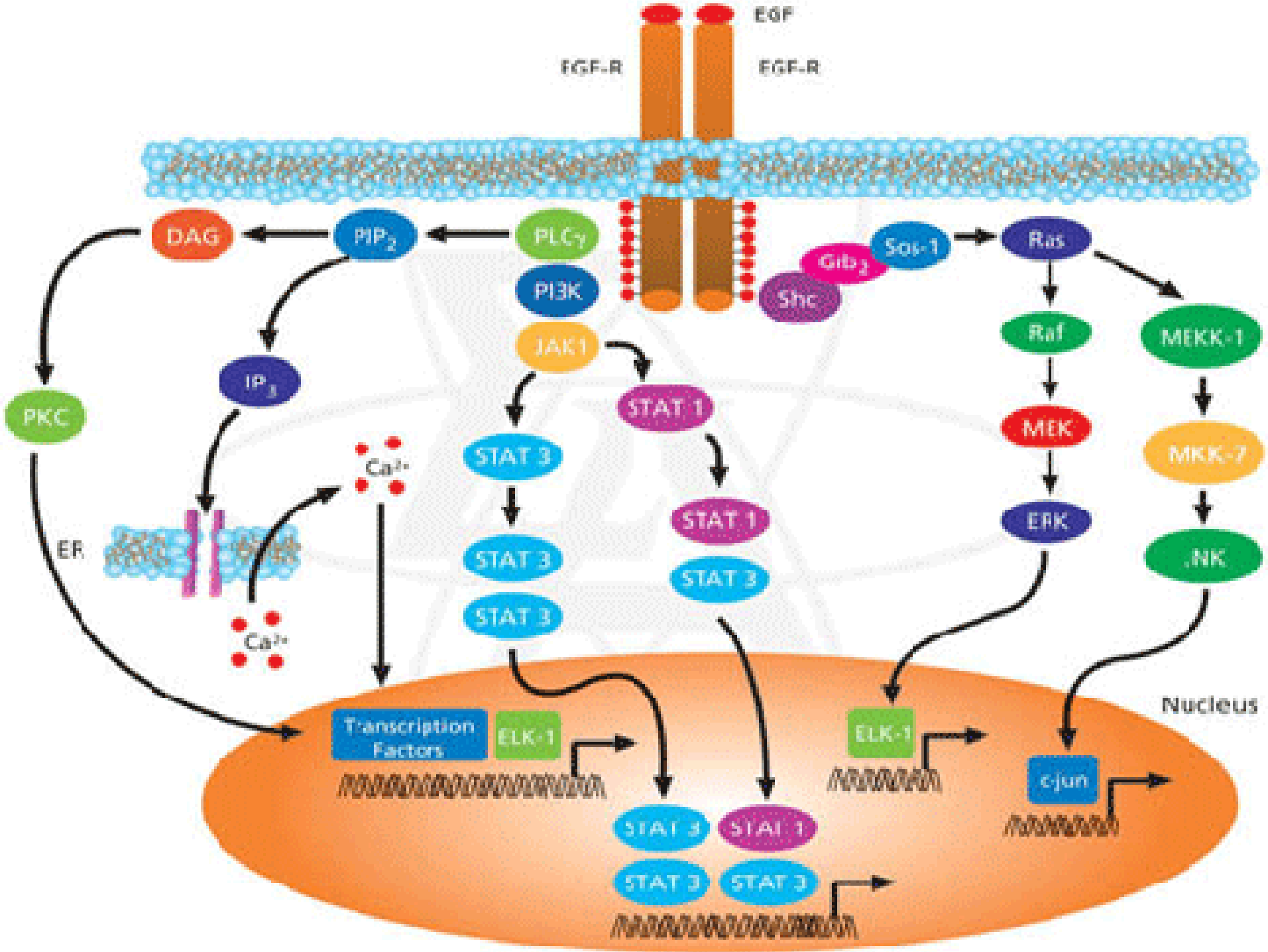

a Normal tissues
c-RET
N
TM
TK
C
Ligand (GDNF family)
RET receptor complex
Activation in presence of ligand
Tyrosine phosphorylation
Regulated growth and survival signals
b Thyroid C-cell carcinogenesis
X Germ-line mutation near TM (e.g. 634) in MEN IIa
● Mutation in TK (e.g. 918): germ-line mutation in MEN IIb, somatic in sporadic cancers
Activation in absence of ligand
Tyrosine phosphorylation
Dysregulated growth and survival signals
C-cell hyperplasia (germ-line mutation); medullary carcinoma of the thyroid (in both germ-line and somatic mutations)
c Thyroid follicular-cell carcinogenesis
Inversion
H4
RET TK
RET–PTC1
Translocation
Rla
RET–PTC2
Inversion
ELE1
RET–PTC3
Activation in absence of ligand
Tyrosine phosphorylation
Dysregulated growth and survival signals
Papillary carcinoma of the thyroid

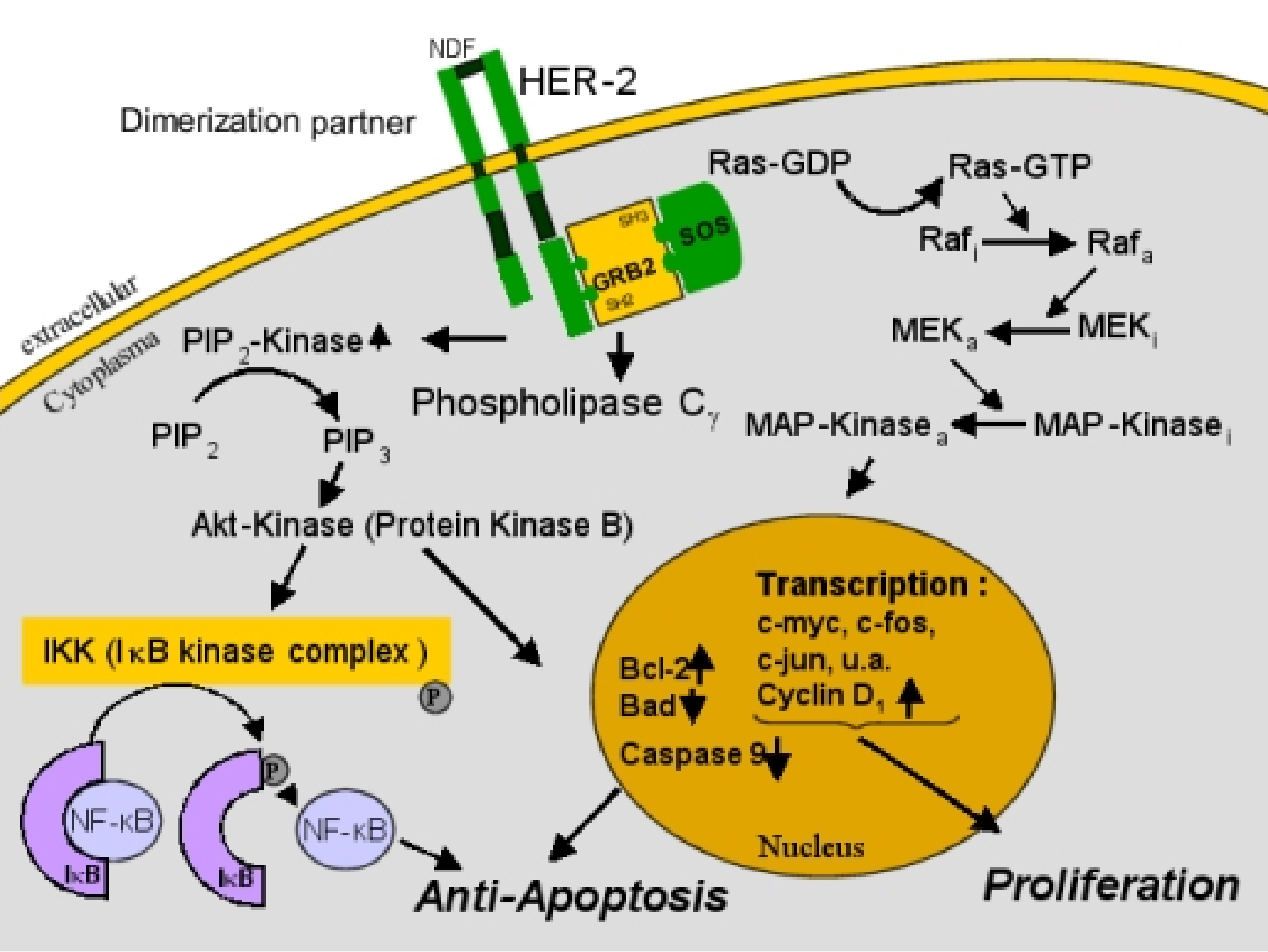
Follicular carcinoma

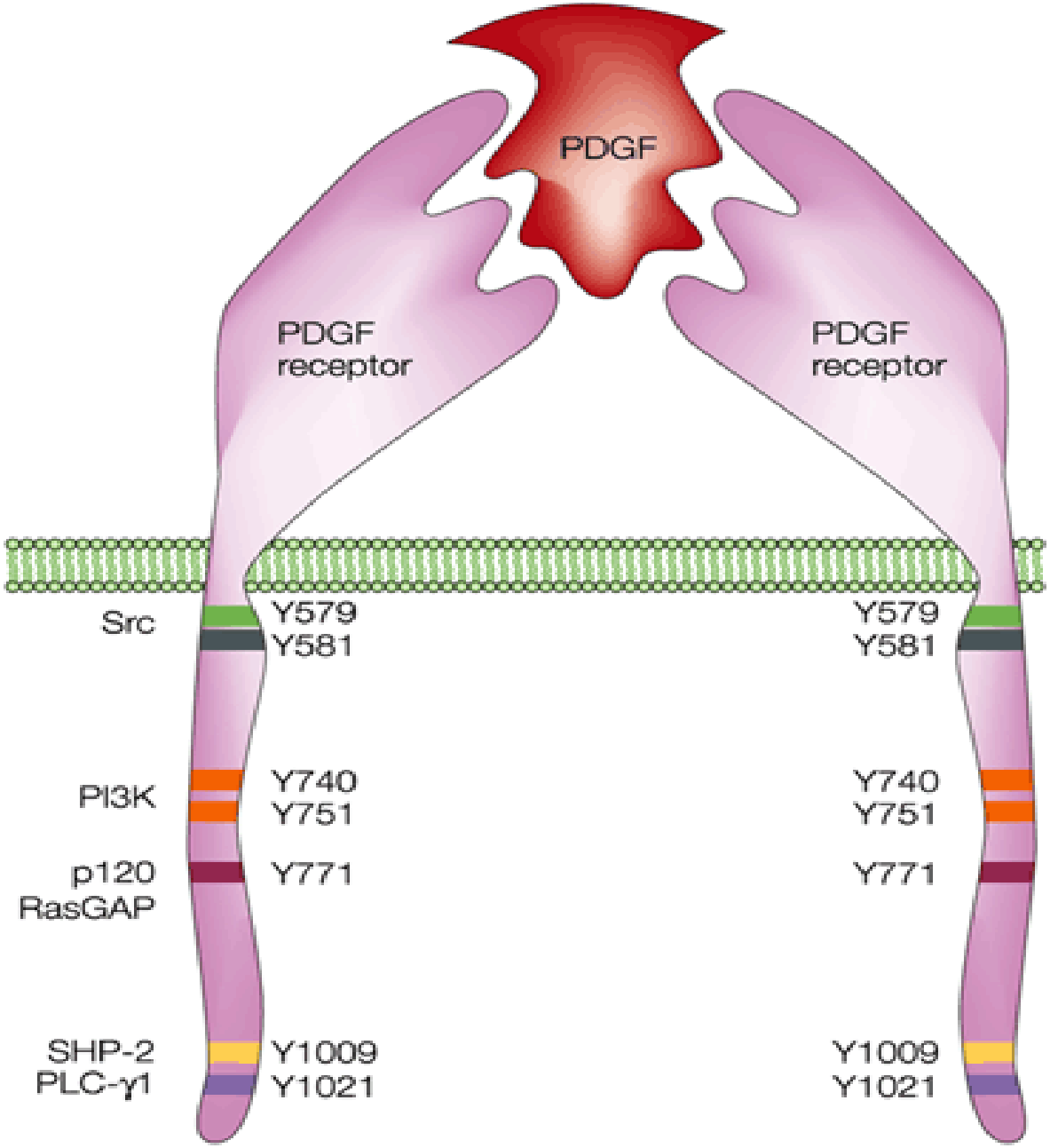
- Ras oncogenes activated
- Deletions chromosome 3p

Anaplastic carcinoma

- No ret activation
- Increased expression of ras, erb-b2, EGF-r
- PDGF-r with autocrine loop PDGF
- Growth factors: TGF-alpha, FGF


EGF
EGF-R
EGF-R
DAG
PIP2
PLCγ
PI3K
JAK1
Grb2
Sos-1
Shc
Ras
Raf
MEK
ERK
MEKK-1
MKK-7
JNK
IP3
PKC
ER
Ca2+
Ca2+
STAT 1
STAT 3
STAT 3
STAT 3
STAT 1
STAT 3
Transcription Factors
ELK-1
ELK-1
c-jun
Nucleus
STAT 3
STAT 1
STAT 3
STAT 3


NDF
HER-2
Dimerization partner
Ras-GDP
Ras-GTP
SH3
SOS
GRB2
SH2
Raf$_i$
Raf$_a$
extracellular
Cytoplasma
PIP$_2$-Kinase
MEK$_a$
MEK$_i$
PIP$_2$
PIP$_3$
Phospholipase C$_\gamma$
MAP-Kinase$_a$
MAP-Kinase$_i$
Akt-Kinase (Protein Kinase B)
Transcription :
c-myc, c-fos,
c-jun, u.a.
Cyclin D$_1$
IKK (IκB kinase complex)
P
Bcl-2
Bad
Caspase 9
NF-κB
IκB
P
NF-κB
IκB
Nucleus
Anti-Apoptosis
Proliferation



Nature Reviews | Molecular Cell Biology

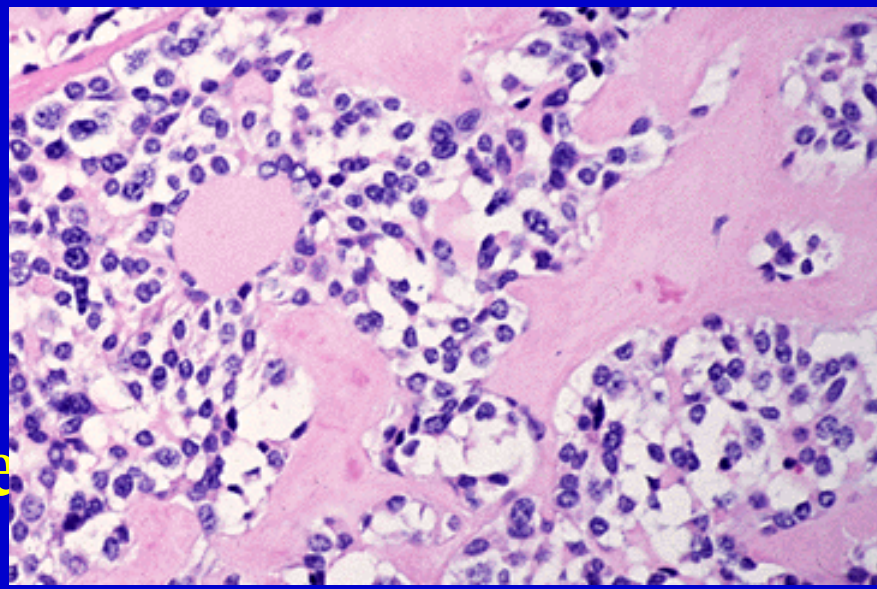
Table 17.3 The multiple endocrine neoplasia (MEN) syndromes, their characteristic tumors and associated genetic abnormalities[a]

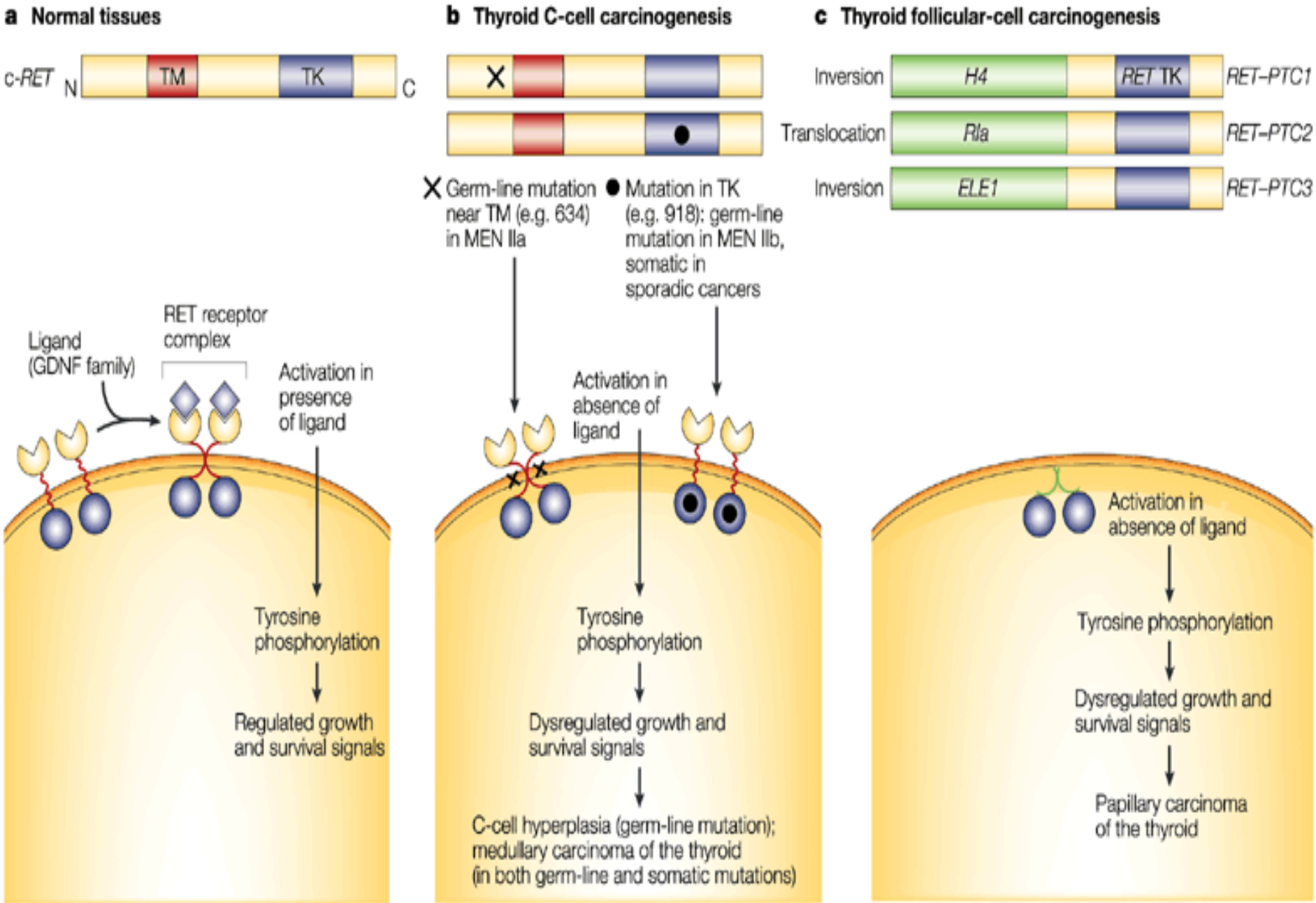
Type (chromosomal location)	Tumors	Gene: most frequently (%) mutated codons[b]
MEN1 (11q13)	Parathyroids	*MEN1:*
	Pancreatic islets:	83/84, 4 bp del (≈10%)
	Gastrinoma	209–211, 4 bp del (≈10%)
	Insulinoma	514–516, del or ins (≈5%)
	Glucagonoma	
	VIPoma	
	PPoma	
	Pituitary (anterior):	
	Prolactinoma	
	Somatotrophinoma	
	Corticotrophinoma	
	Non-functioning	
	Associated tumors:	
	Adrenal cortical	
	Carcinoid	
	Lipoma	
	Angiofibromas	
	Collagenomas	
MEN2 (10 cen–10q.11.2)		
MEN2a	Medullary thyroid carcinoma (MTC)	*ret:* 634, missense, e.g. Cys → Arg (≈85%)
	Pheochromocytoma	
MTC-only	Medullary thyroid carcinoma (MTC)	*ret:* 618, missense (>50%)
MEN2b	Medullary thyroid carcinoma (MTC)	*ret:* 918, Met → Thr (>95%)
	Pheochromocytoma	
	Parathyroid	
	Associated abnormalities:	
	Mucosal neuromas	
	Marfanoid habitus	
	Medullated corneal nerve fibers	
	Megacolon	

Autosomal dominant inheritance of the MEN syndromes has been established.
[a] Reproduced with permission from Thakker.[2]
[b] del, deletion; ins, insertion.

Medullary carcinoma

- Ret proto-oncogene
- MEN type 2 syndrome




a Normal tissues
c-RET
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H4
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Translocation
Rla
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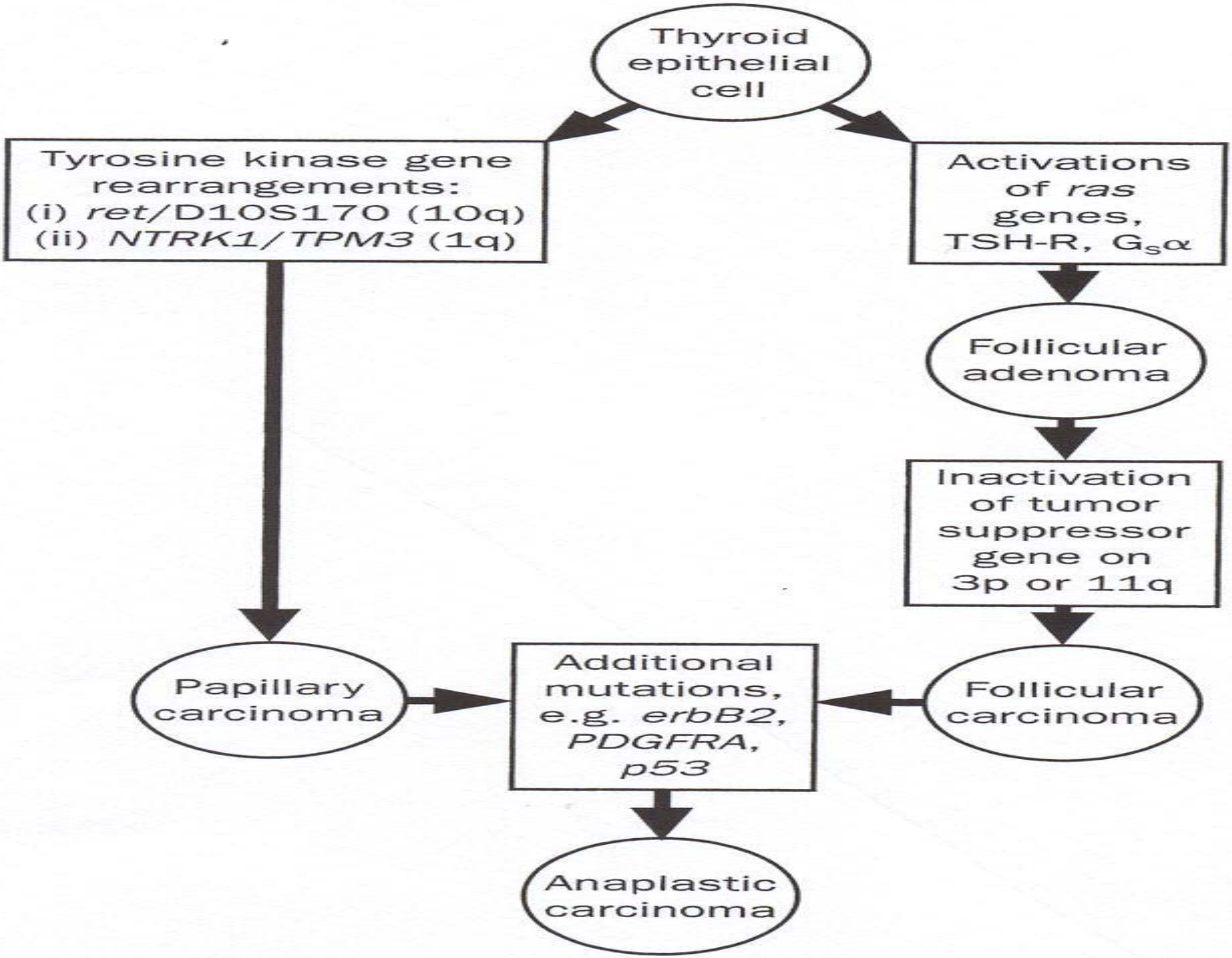
Inversion
ELE1
RET–PTC3
Activation in absence of ligand
Tyrosine phosphorylation
Dysregulated growth and survival signals
Papillary carcinoma of the thyroid


Thyroid epithelial cell
Tyrosine kinase gene rearrangements:
(i) ret/D10S170 (10q)
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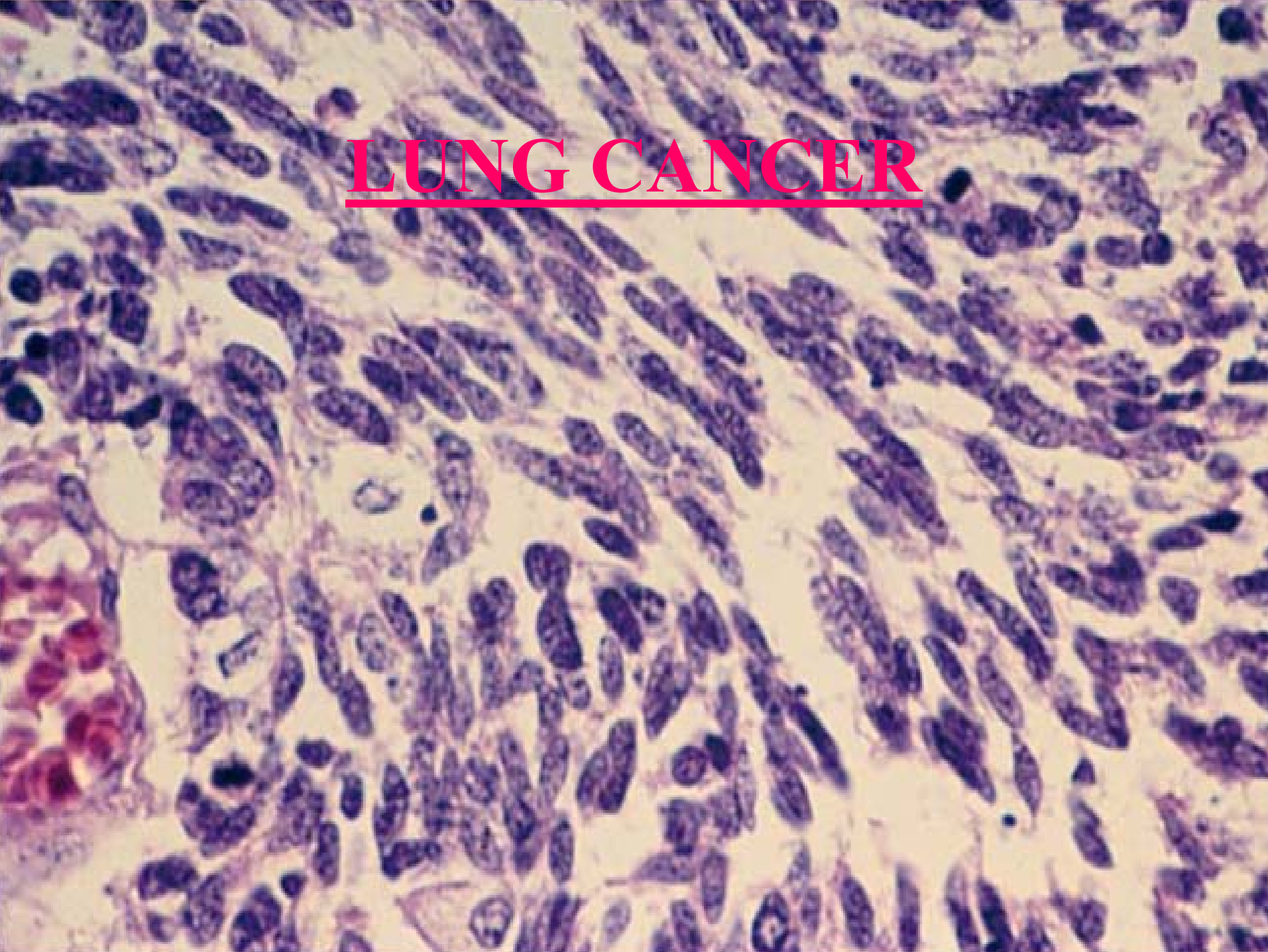
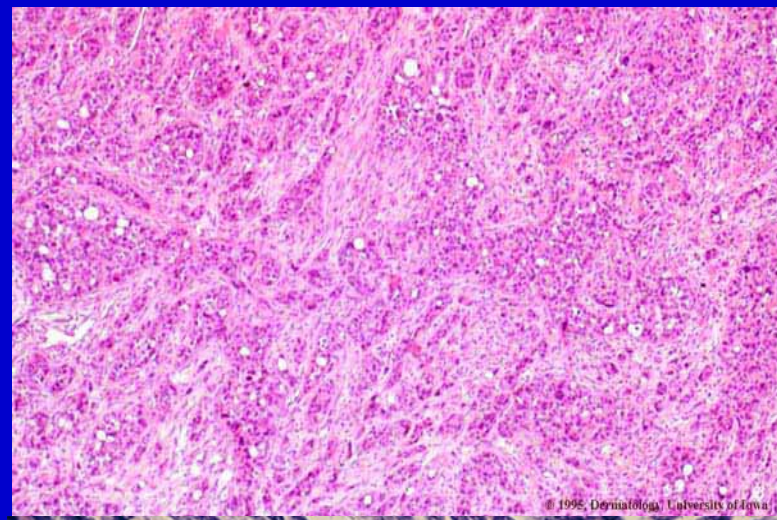
Activations of ras genes, TSH-R, $G_s\alpha$
Follicular adenoma
Inactivation of tumor suppressor gene on 3p or 11q
Papillary carcinoma
Additional mutations, e.g. erbB2, PDGFRA, p53
Follicular carcinoma
Anaplastic carcinoma


LUNG CANCER

- **Non-small cell types**
- **Small cell types**



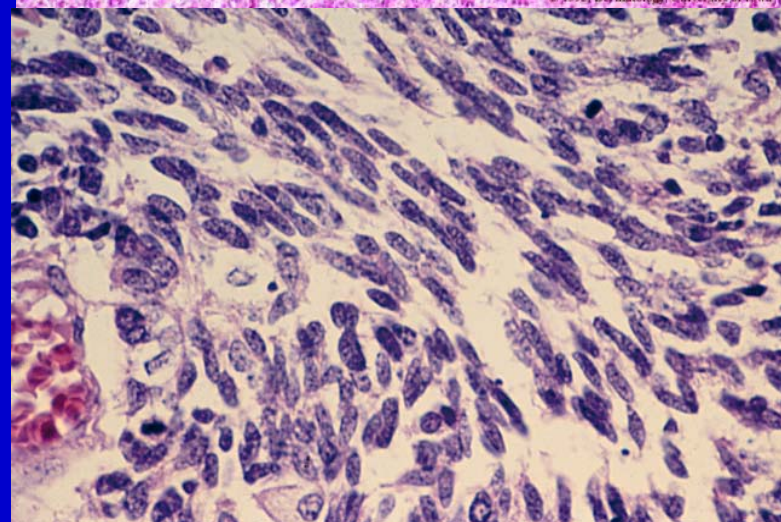


Table 19.1 Frequency of cell types of lung cancer[1]

Non-small cell carcinomas:	
Adenocarcinoma	30%
Squamous cell carcinoma	35%
Large cell carcinoma	15%
Small cell carcinoma	20%

SCLC

- Myc oncogene amplification
- X4 after chemotherapy

NSCLC

- P53: diagnosis, prognosis and gene therapy
- K-ras mutations
- Bcl-2 : good prognosis – poor prognosis ?
- EGF-receptor:
  chemotherapy respons: resistance to doxorubicin

Table 19.4 Proposed molecular markers of poor prognosis in lung cancer

p53
Rb
K-Ras/p21
Myc
c-ErbB-2
p16
Cyclin D
Cyclin E
Bcl-2/Bax
FUC-T IV and VII

Table 19.3 Positive p53 immunostaining in pleural biopsies

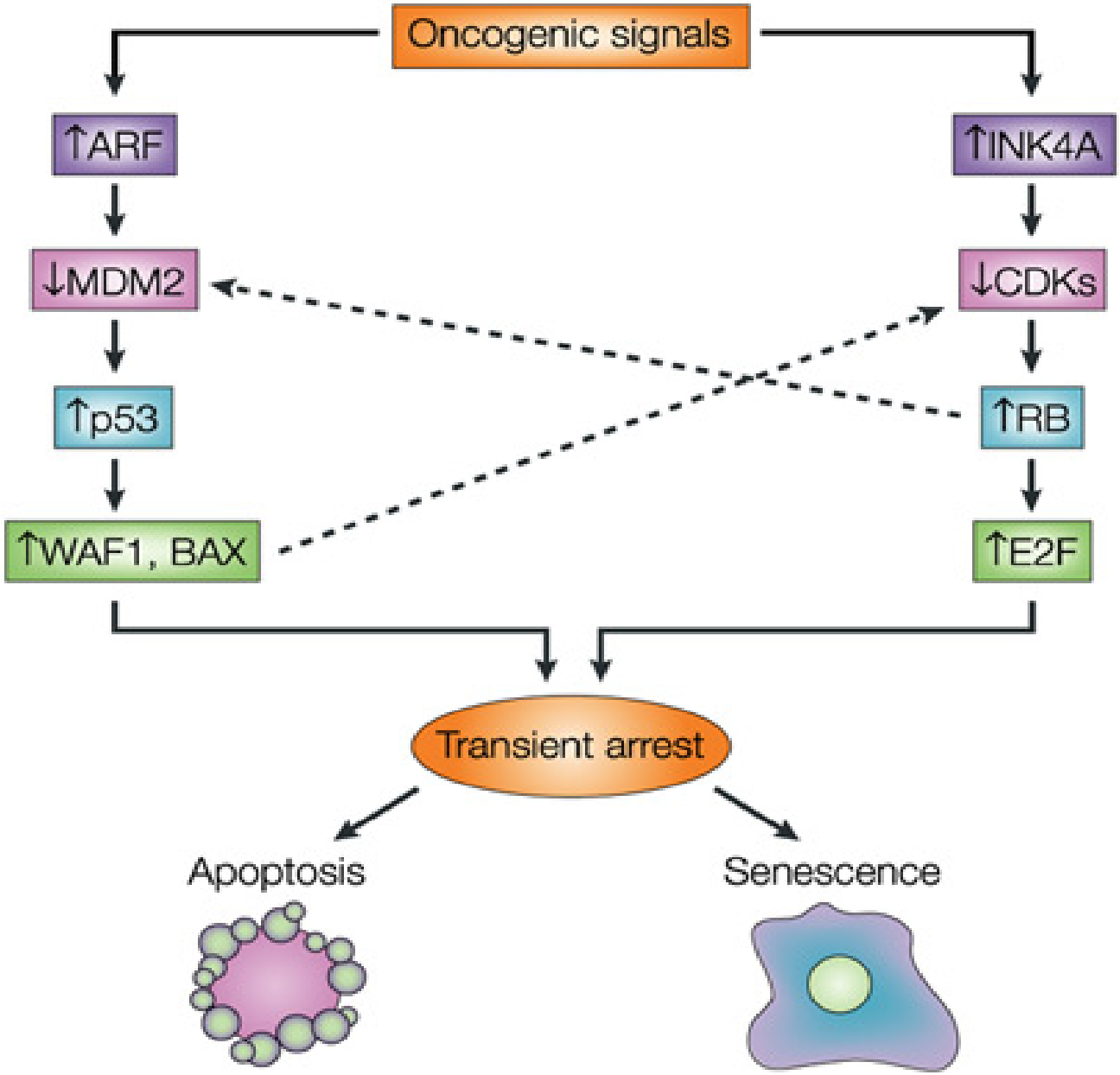
	Number positive/total		
Study	Metastatic adenocarcinoma	Mesothelioma	Reactive atypia
Cagle et al[74]	11/20	19/40	0/13
Kafiri et al[75]		14/20	0/20
Mayall et al[76]		21/47	0/20
Ramael et al[77]		9/36	0/20

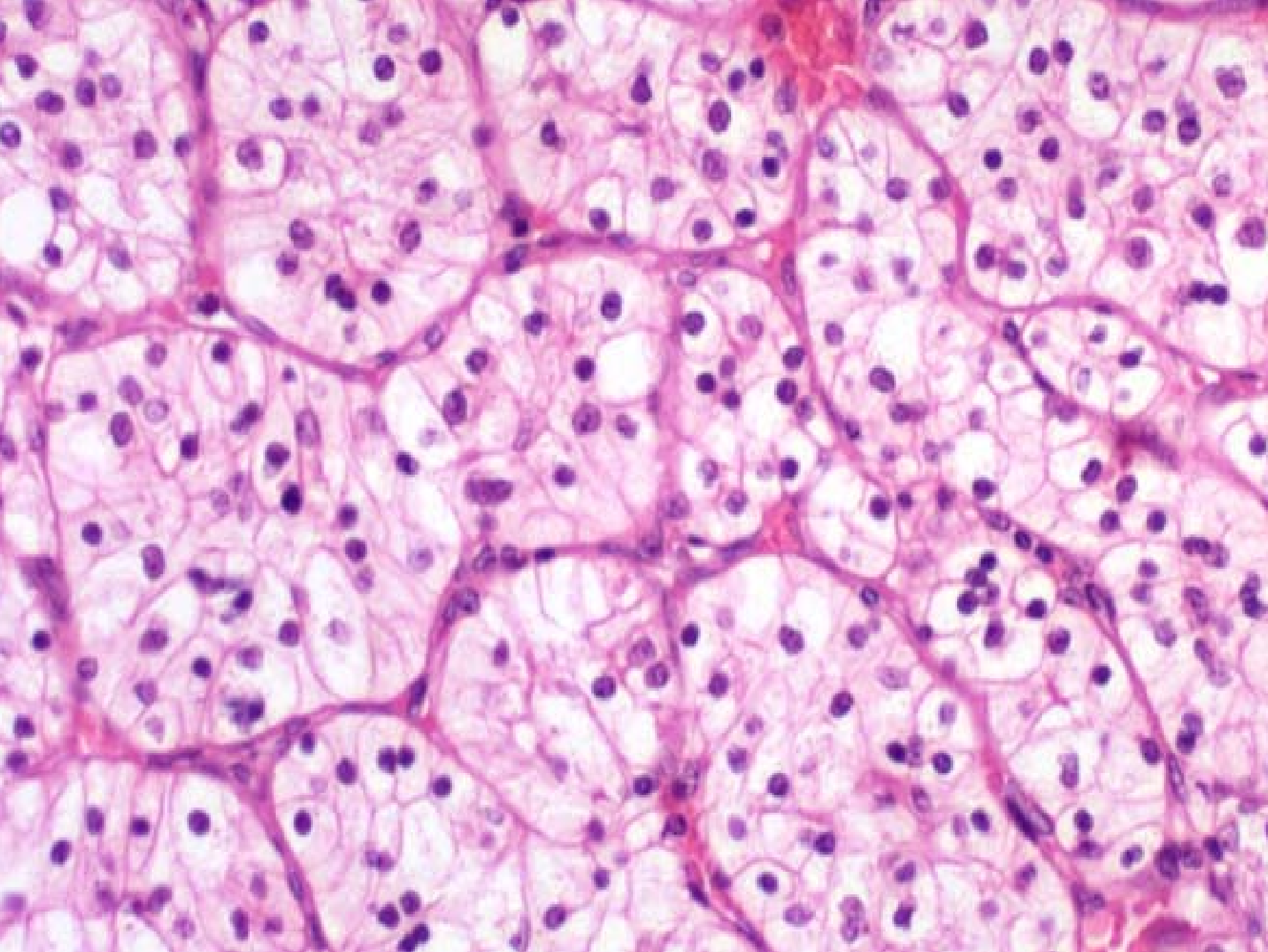
RENAL CELL TUMOURS

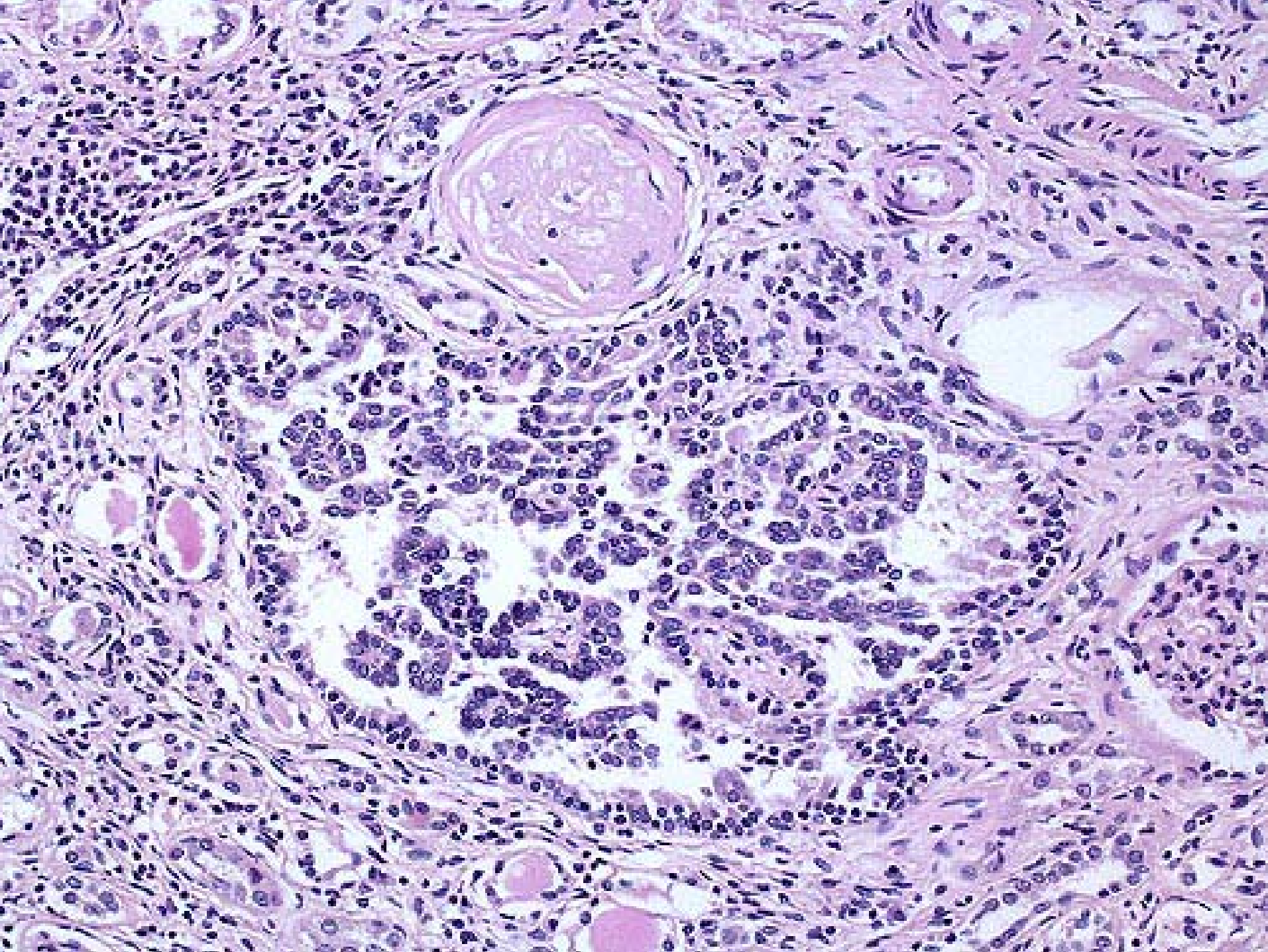
- **7/ 100. 000 inhabitants**
- **Peak incidence decade 6**
- **40% metastasized at diagnosis**
- **Genetic dd system described**

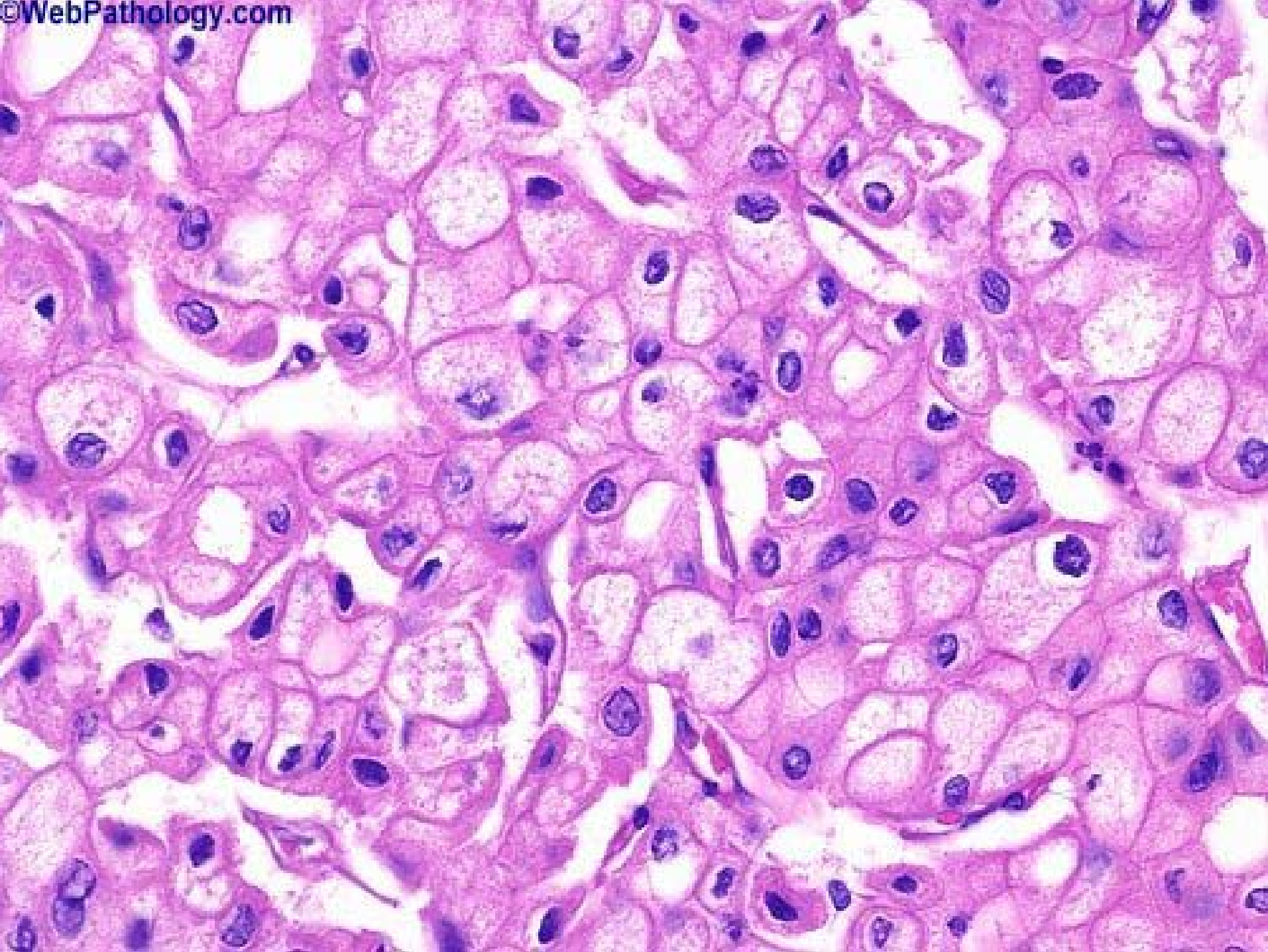
Pathology

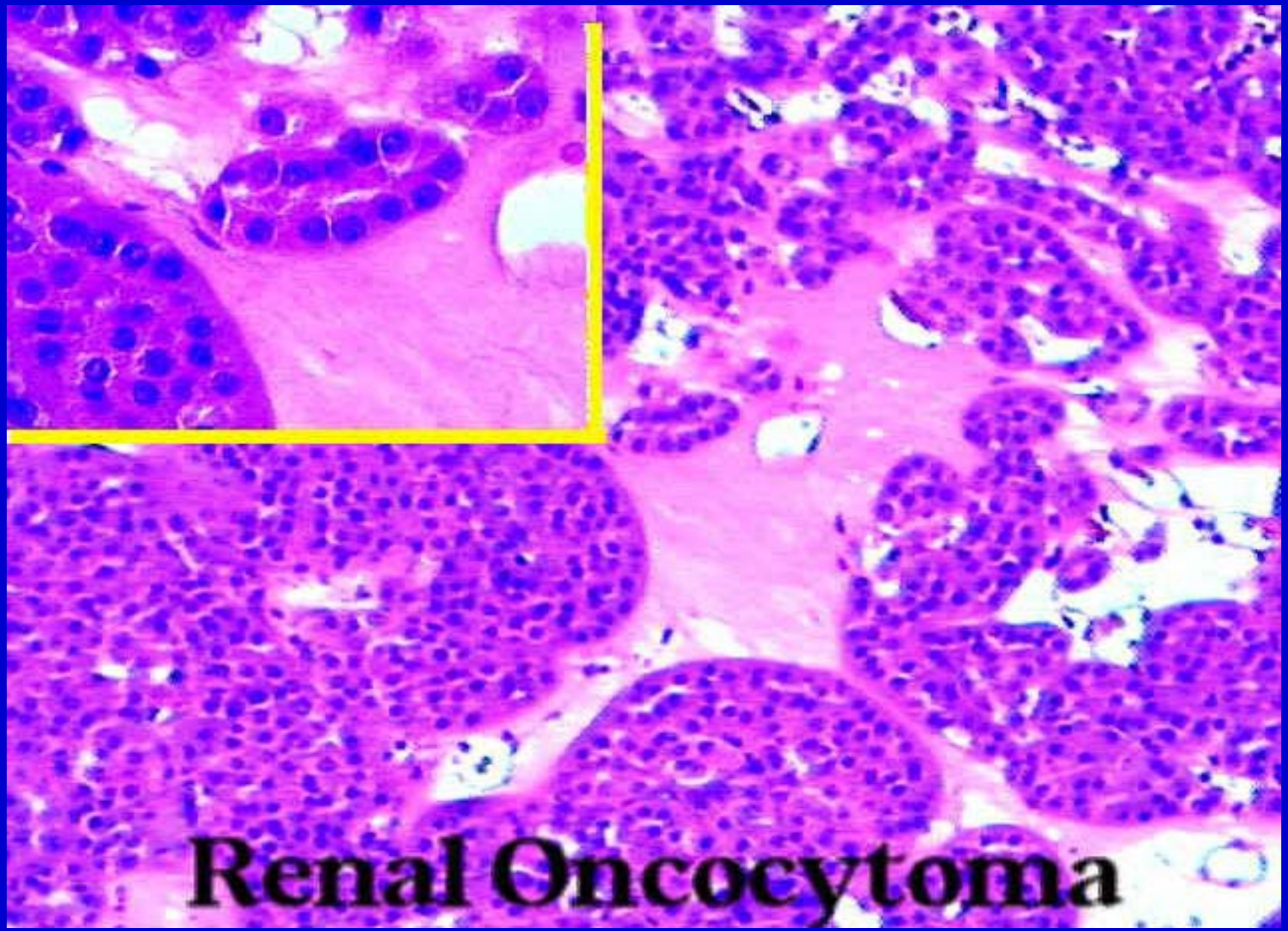
- Non-papillary type 80%
- Papillary type 10%
- Chromophobe type
- Renal oncocytoma

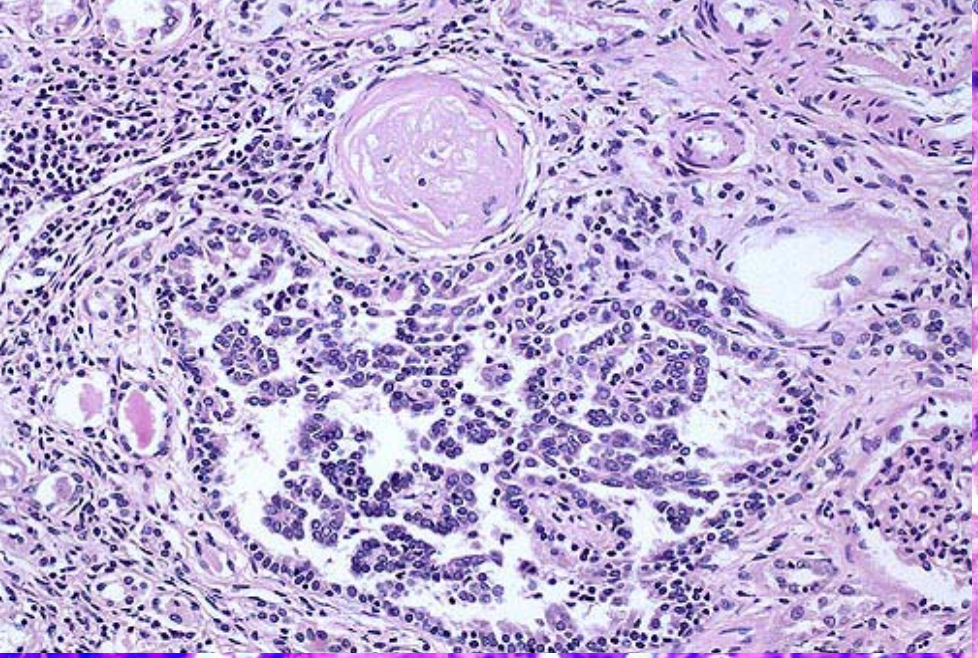

Oncogenic signals
↑ARF
↑INK4A
↓MDM2
↓CDKs
↑p53
↑RB
↑WAF1, BAX
↑E2F
Transient arrest
Apoptosis
Senescence

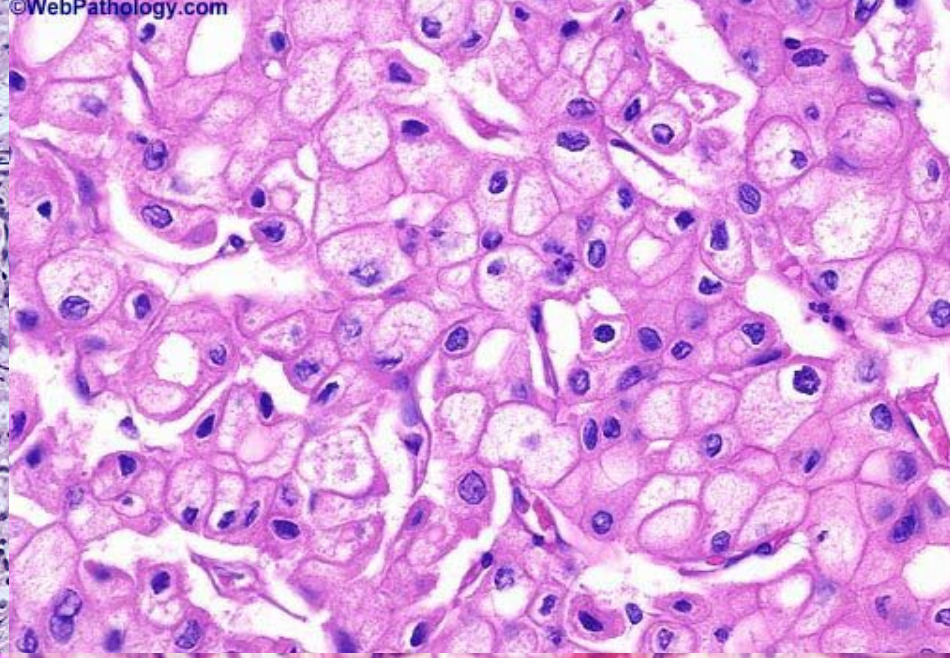







Renal Oncocytoma

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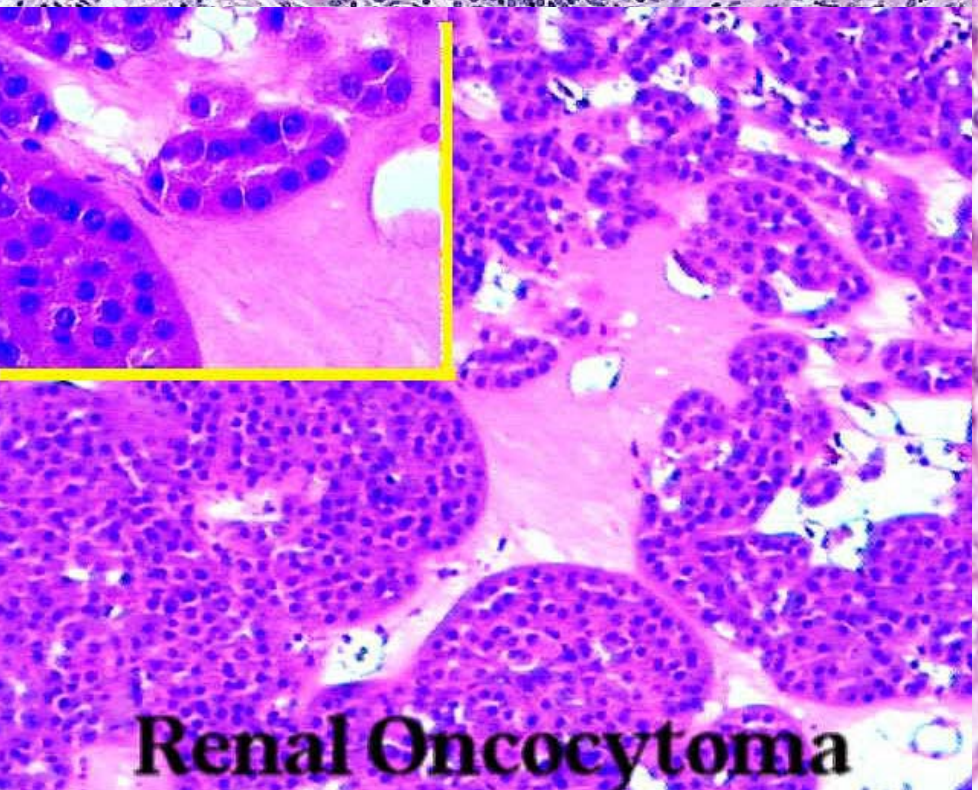
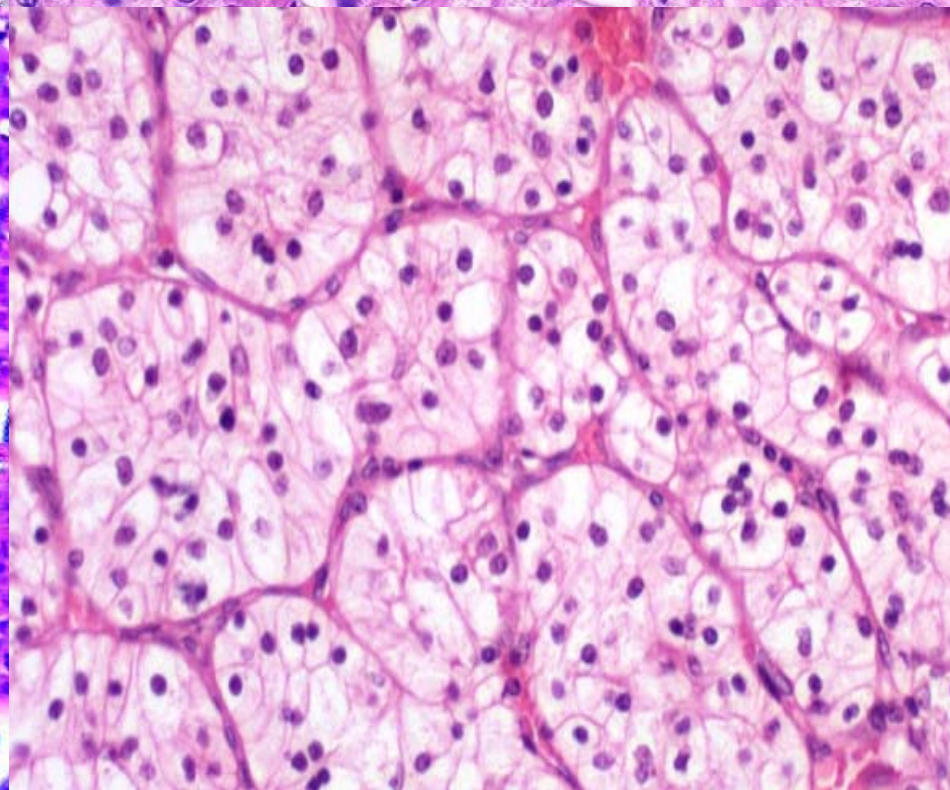
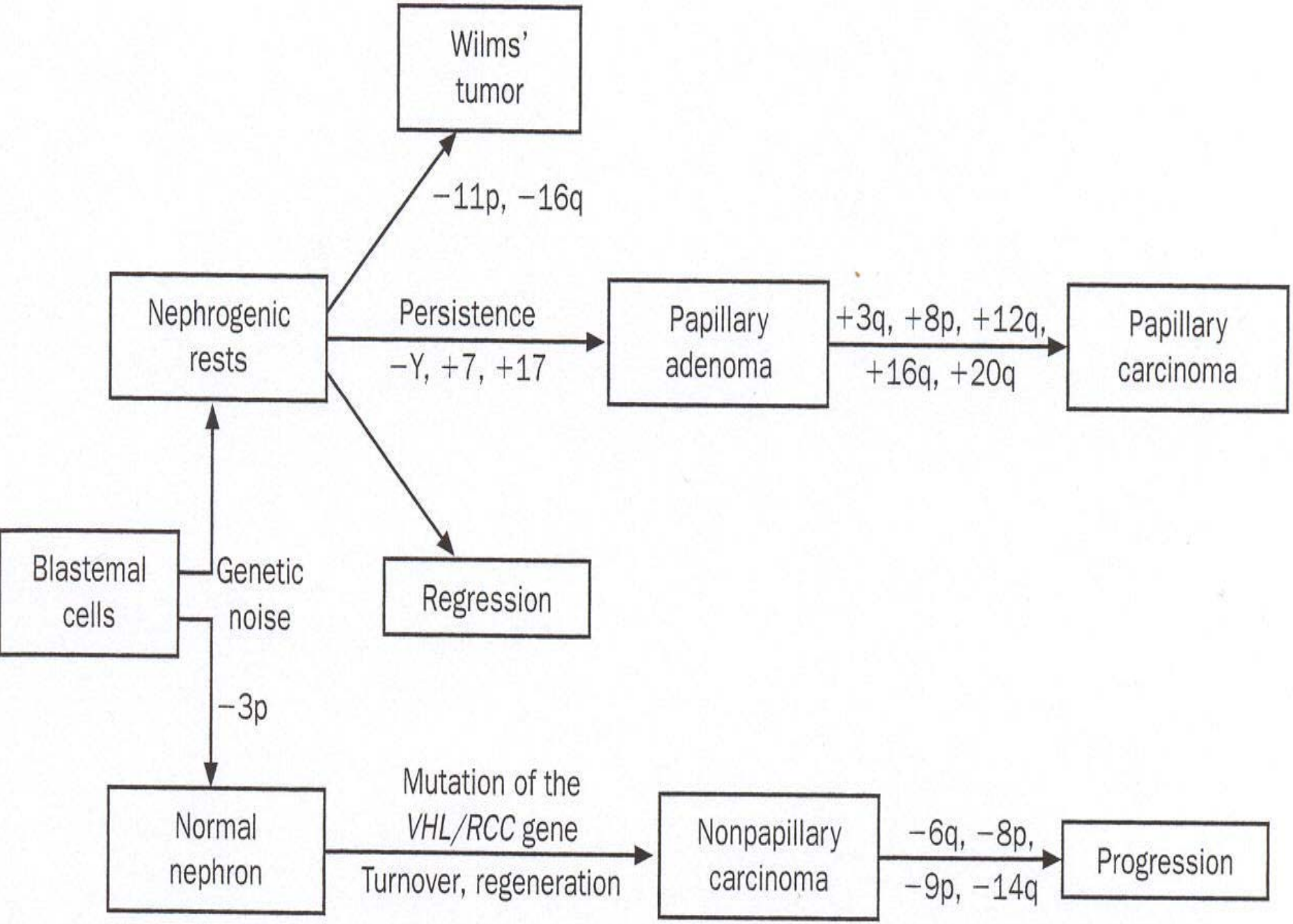

Renal Oncocytoma



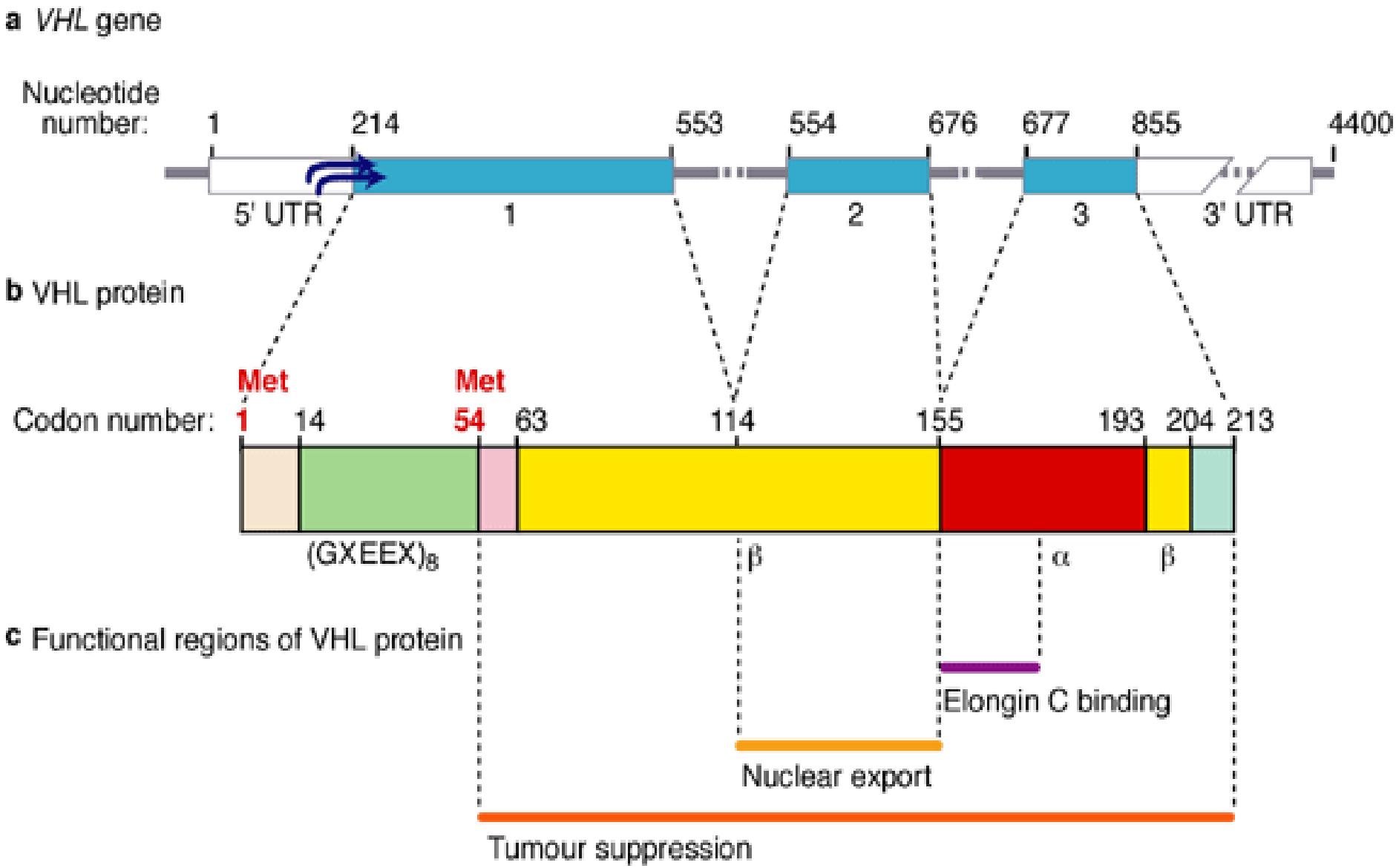

Wilms' tumor
−11p, −16q
Nephrogenic rests
Persistence
−Y, +7, +17
Papillary adenoma
+3q, +8p, +12q,
+16q, +20q
Papillary carcinoma
Blastemal cells
Genetic noise
Regression
−3p
Normal nephron
Mutation of the VHL/RCC gene
Turnover, regeneration
Nonpapillary carcinoma
−6q, −8p,
−9p, −14q
Progression

Non papillary RCC

- 3 p deletion : suppressor gene 97%

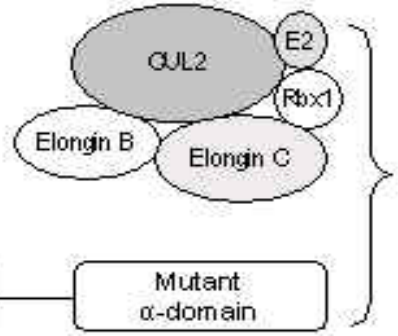
- VHL gene at 3p25(tumour suppressor gene)
- Development renal cysts, non-papillary RCC, hemangioblastoma and pheochromocytoma

- Other suppressor genes at 3p !!



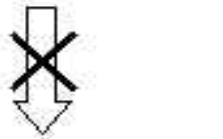
von Hippel–Lindau (*VHL*) gene and protein structure and function

VHL Gene Mutation

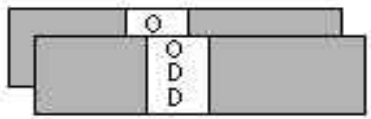


VHL Complex Disrupted

VHL Protein



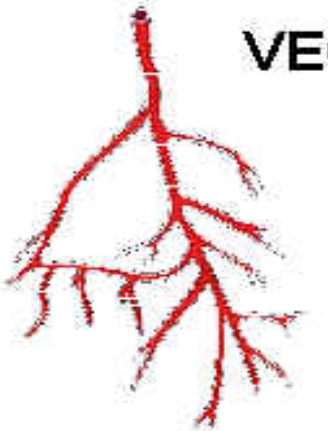
HIF1-α HIF2-α Accumulation

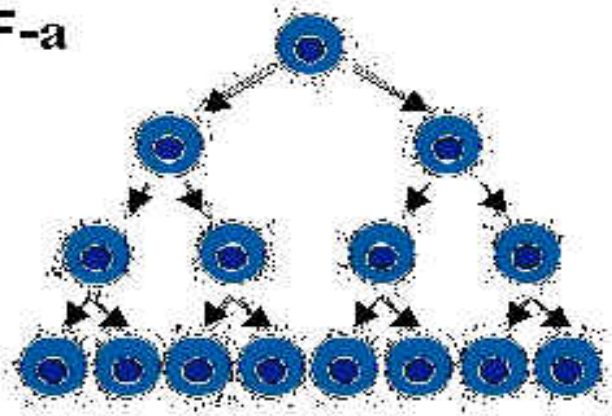


VEGF

Glut-1

TGF-a

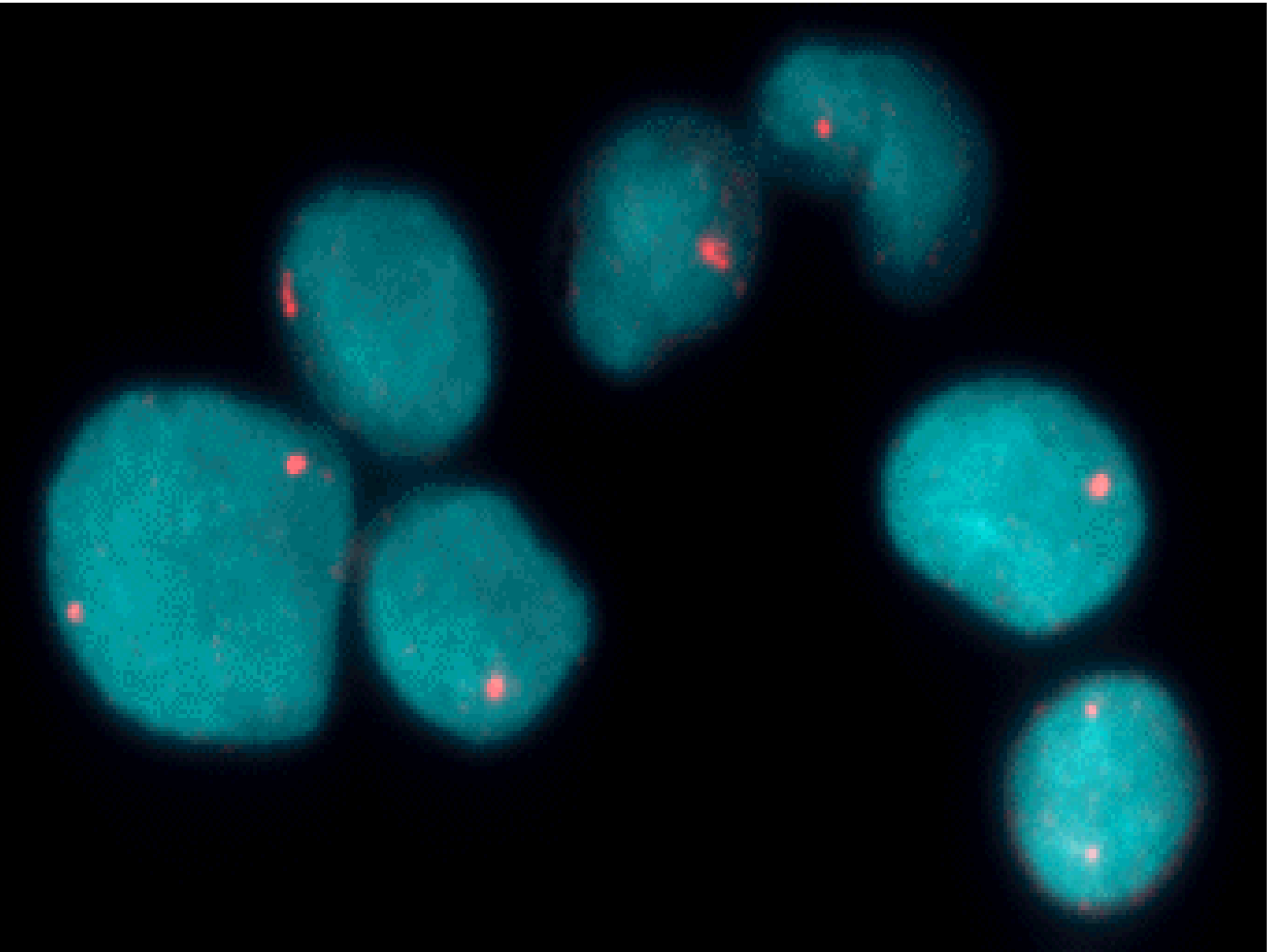




Angiogenesis

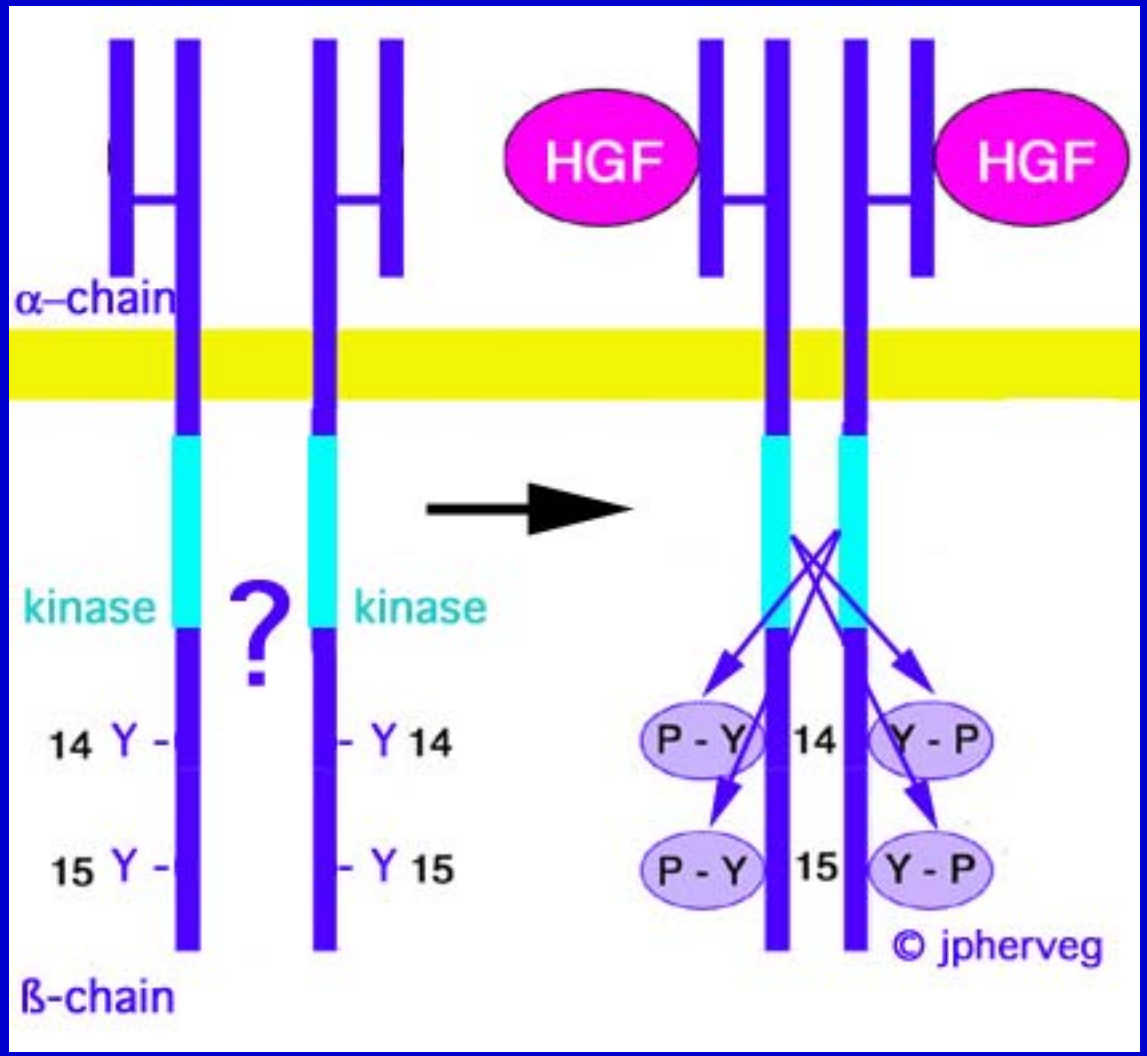
Glucose Transport

Autocrine Growth Stimulation



Papillary RCC

- **Trisomies 7, 17, 3q, 8p, 12q, 16q, 20 q**
- **Missense mutation in MET gene (cytoplasmic domain tyrosine kinase) leading to overexpression of protein**


HGF
HGF
α–chain
kinase
?
kinase
14 Y -
- Y 14
15 Y -
- Y 15
P - Y
14
Y - P
P - Y
15
Y - P
© jpherveg
ß-chain

Chromofobe RCC

- **Monosomy at random**
- **LOH of 1p, 2, 6, 10, 13, 17, 21**
- **Rearrangement of mitochondrial DNA**

Renal Oncocytoma

- **Heterogenous genetics**
- **Largest group: normal**
- **Subgroup: LOH 1 and 14, tr 11q13**
- **Genes for mitochondrial enzymes: oxidative phosphorylation**

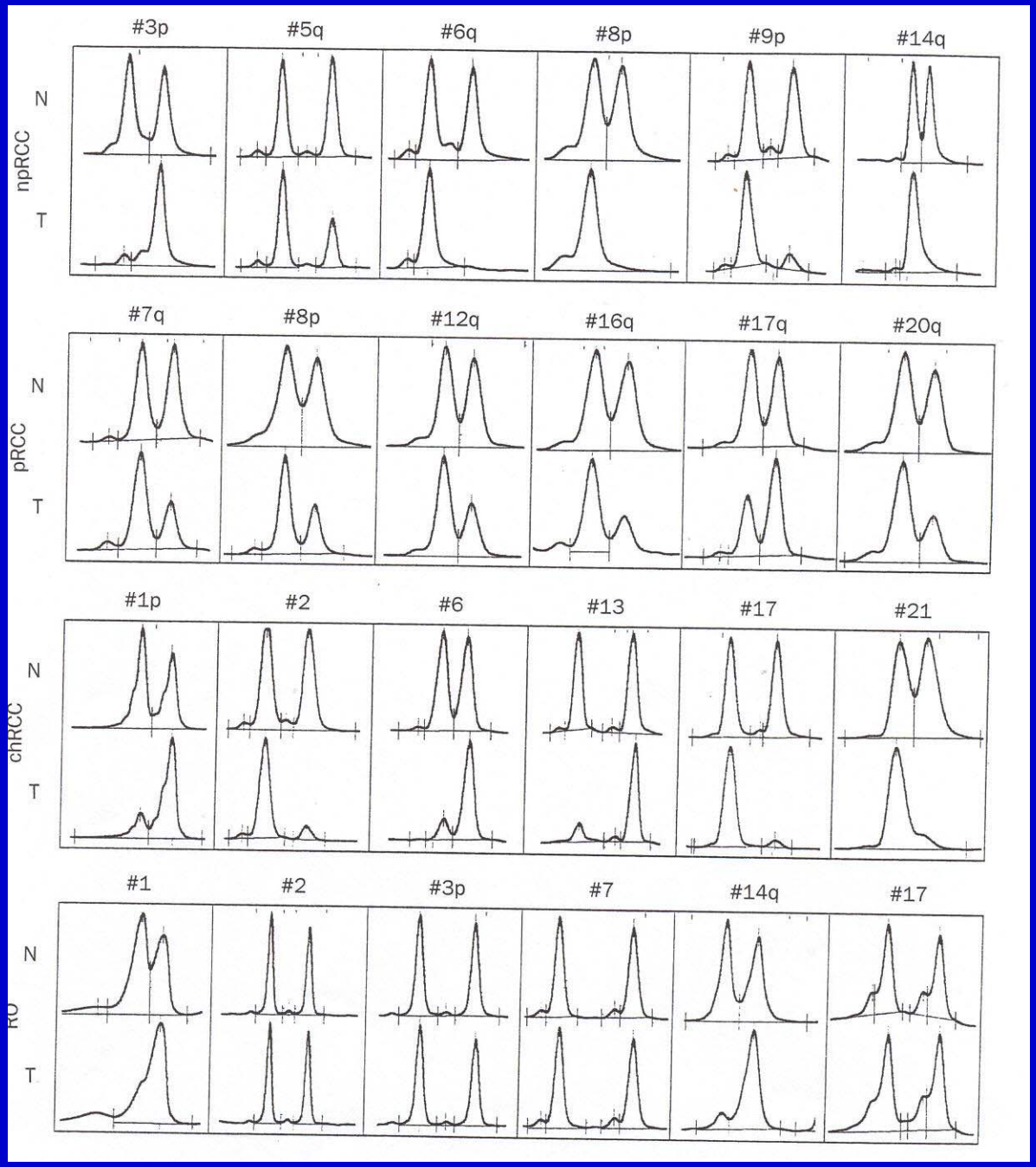

#3p
#5q
#6q
#8p
#9p
#14q
N
T
npRCC
#7q
#8p
#12q
#16q
#17q
#20q
N
T
pRCC
#1p
#2
#6
#13
#17
#21
N
T
chRCC
#1
#2
#3p
#7
#14q
#17
N
T

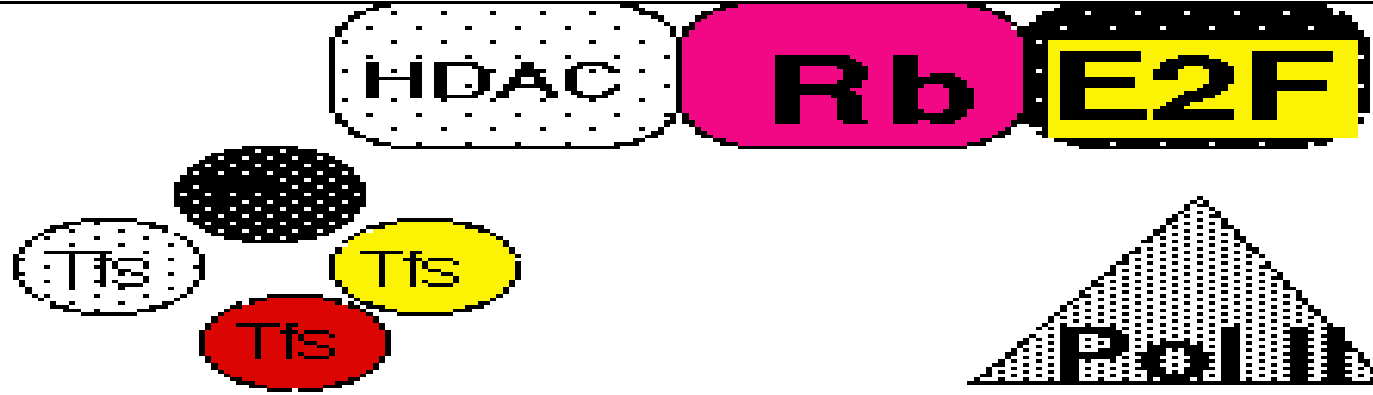
Table 24.1 Differential genetics of renal cell tumors

Type of tumor[a]	Genetic alterations (%)																		
	−Y	+7	+17	+3q	+8	+12	+16	+20	−3p	+5q	−6q	−8p	−9p	−14q	−1p	−2	−6	−10	−13	−17	−21
pRCA	77	100	100	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
pRCC	93	85	87	24	25	42	55	48	—	—	—	—	—	—	—	—	—	—	—	—	—
npRCC	26	15	—	—	—	—	—	—	98	48	23	33	33	47	—	—	—	—	—	—	—
chRCC	—	—	—	—	—	—	—	—	25	—	—	25	18	—	100	96	88	88	96	76	88
RO	t(11q13;?) / −Y, −1, −14 / normal karyotype																				
CDC											?	?	?								

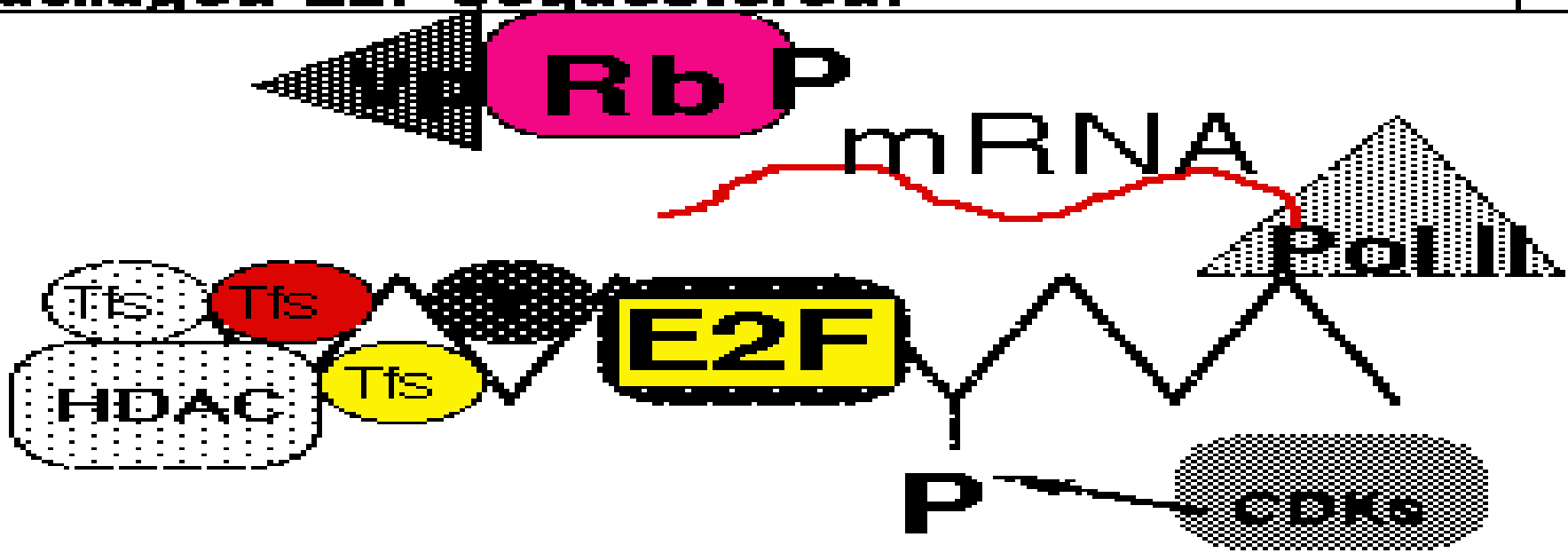
[a] pRCA, papillary renal cell adenoma; pRCC, papillary renal cell carcinoma; npRCC, nonpapillary renal cell carcinoma; chRCC, chromophobe renal cell carcinoma; RO, renal oncocytoma; CDC, collecting duct carcinoma.

Diagnostic applications

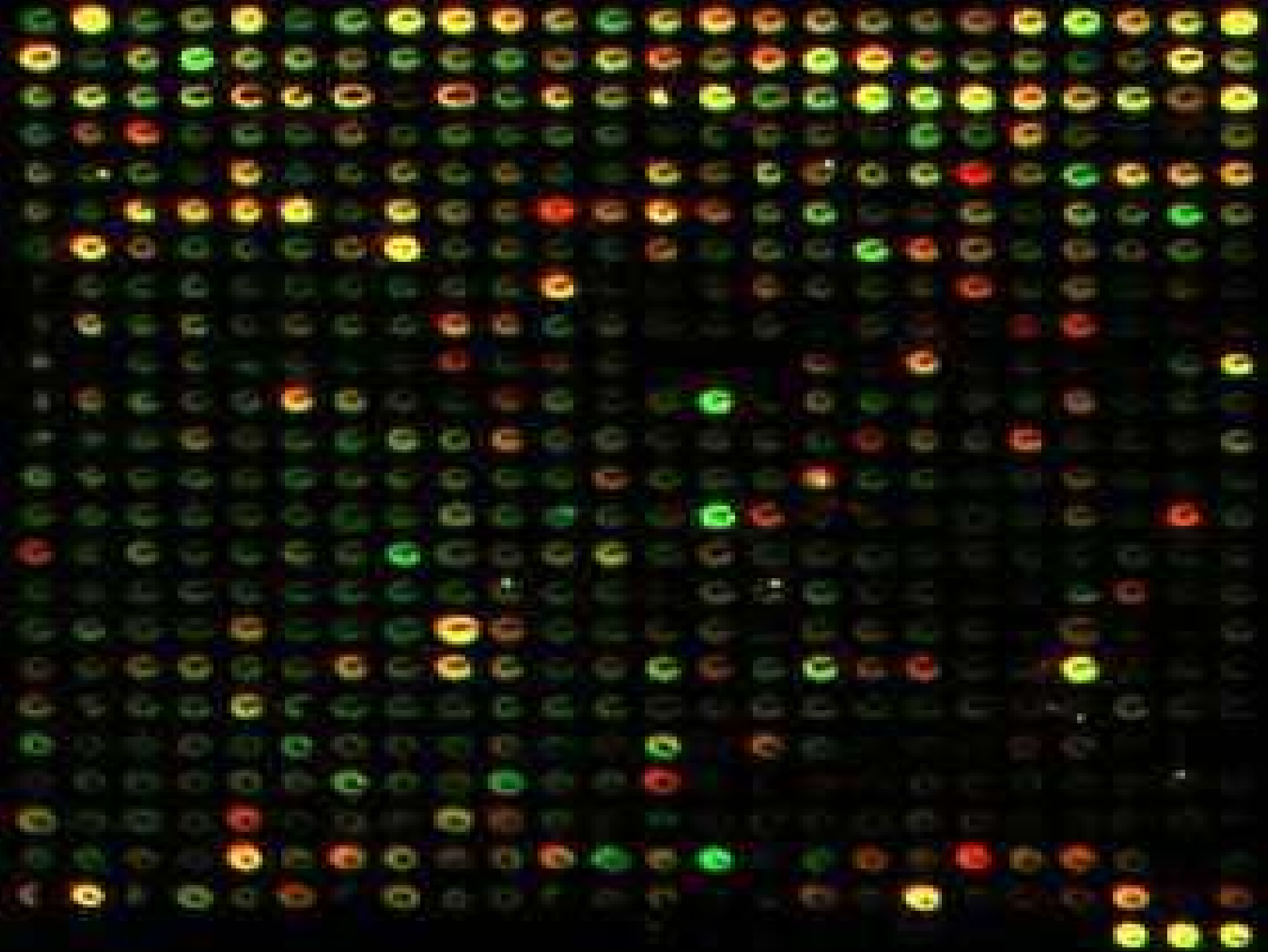
- **Papillary RCC: trisomy 7 and 17**
- **Non-papillary RCC: LOH 3, 6,8,9,14**
- **Renal oncocytoma: absence or LOH 1 and 14**



Histones deacetylated. DNA packaged E2F sequestered.

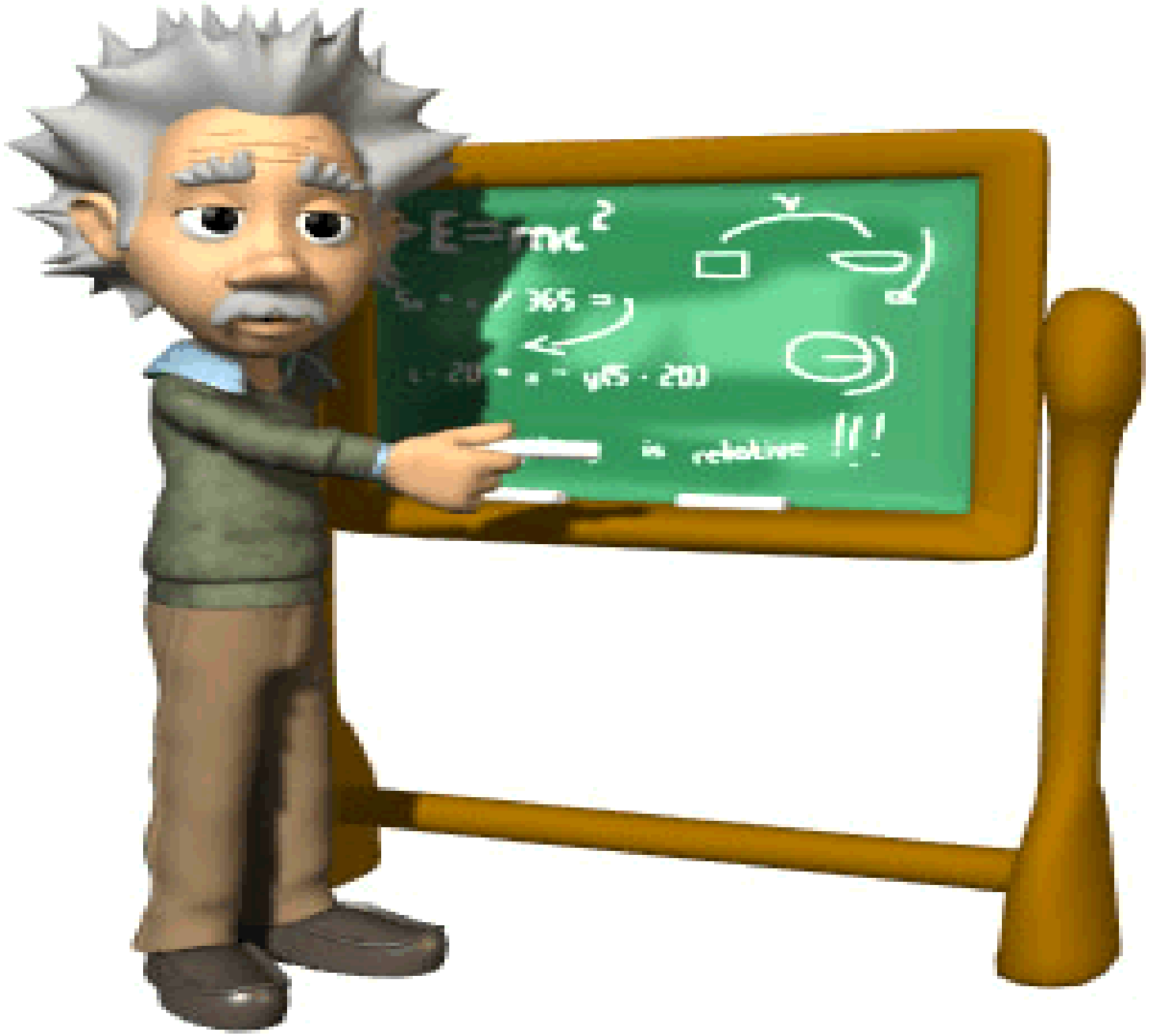


Histones acetylated, DNA phosphorylated and opened up. Transcription factors, pol II recruited.Transcription




MOLECULAR PATHOLOGY:
GARDEN OF EDEN or GATE TO
HELL FOR THE SURGICAL
PATHOLOGIST ?


IT IS UP TO US TO DECIDE
!!


$E=mc^2$
365 =
is relative !!!