L'analisi di mutazioni di RET in Endocrinologia

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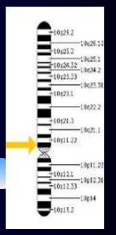
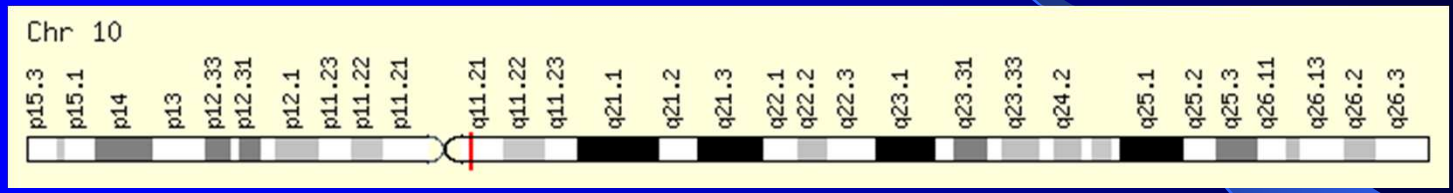
RET mutations in Endocrinology



RET (REarranged during Transfection) protooncogene



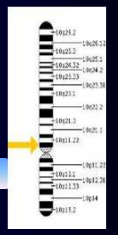
Long arm of chromosome 10
(10q11.2)

susceptibility gene for

- familial medullary thyroid cancer (FMTC)
- pheochromocytoma
- parathyroid hyperplasia/adenomas

multiple endocrine neoplasia type 2 (MEN 2)

RET mutations in Endocrinology

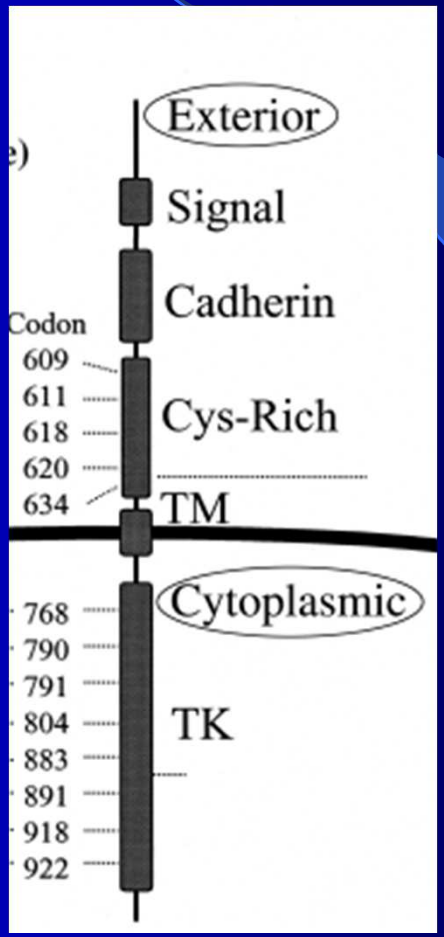


RET ➔ transmembrane receptor

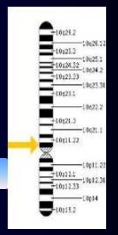
extracellular domain

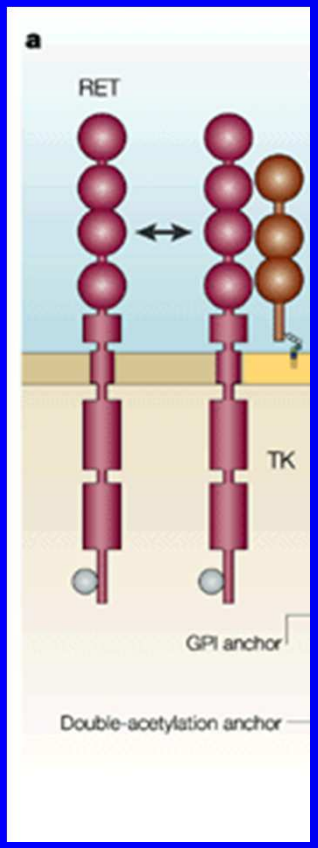
transmembrane domain

tyrosine kinase domain



RET mutations in Endocrinology





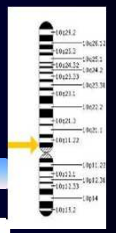
RET
dimerizes

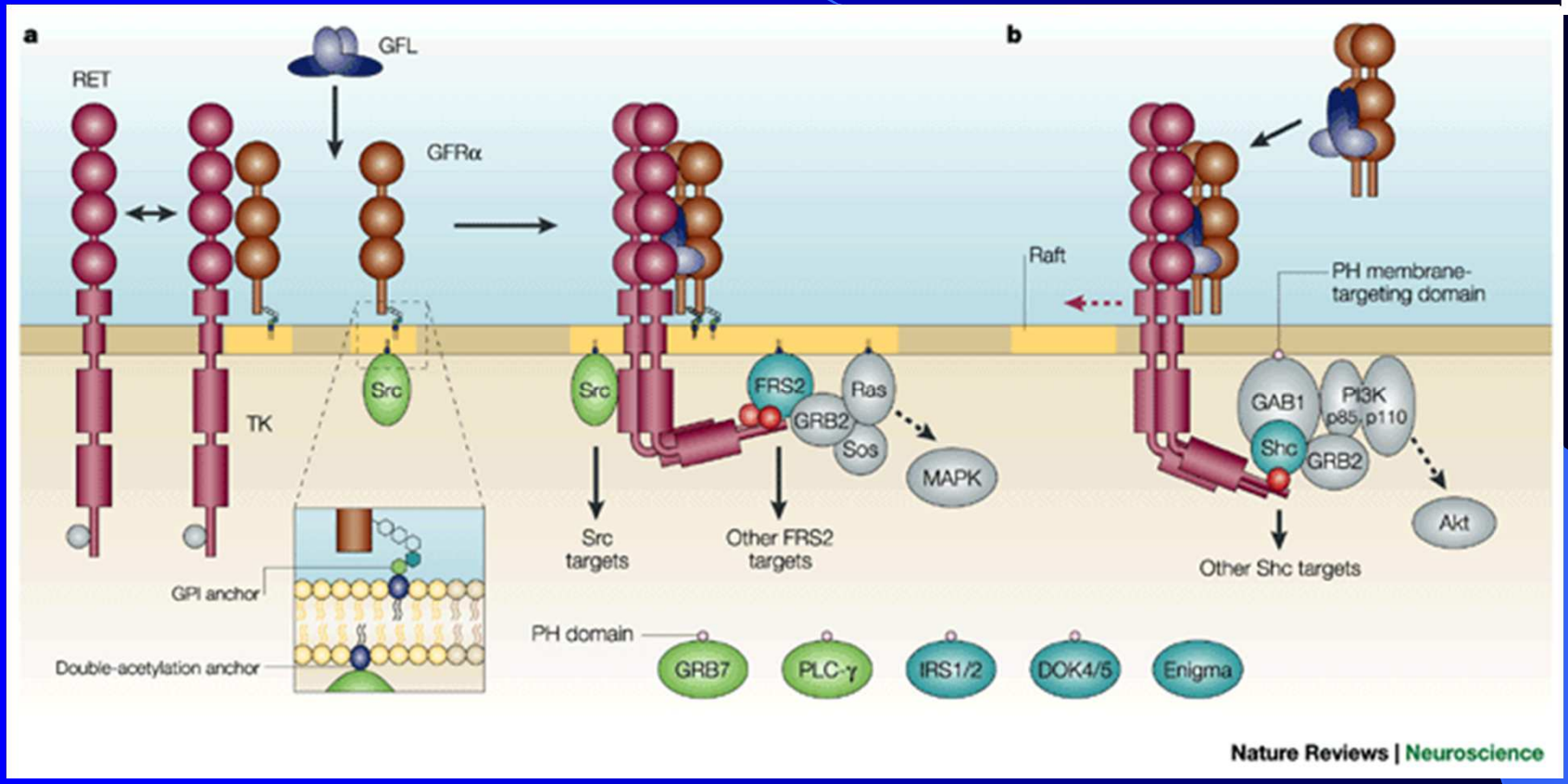
associates with GFR -1

forms the GDNF receptor
(glial cell line-derived neurotrophic factor recepor, GDNF R)

In the absence of GDNF, RET and GFR-1 do not dimerize

RET mutations in Endocrinology



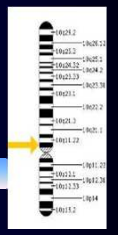


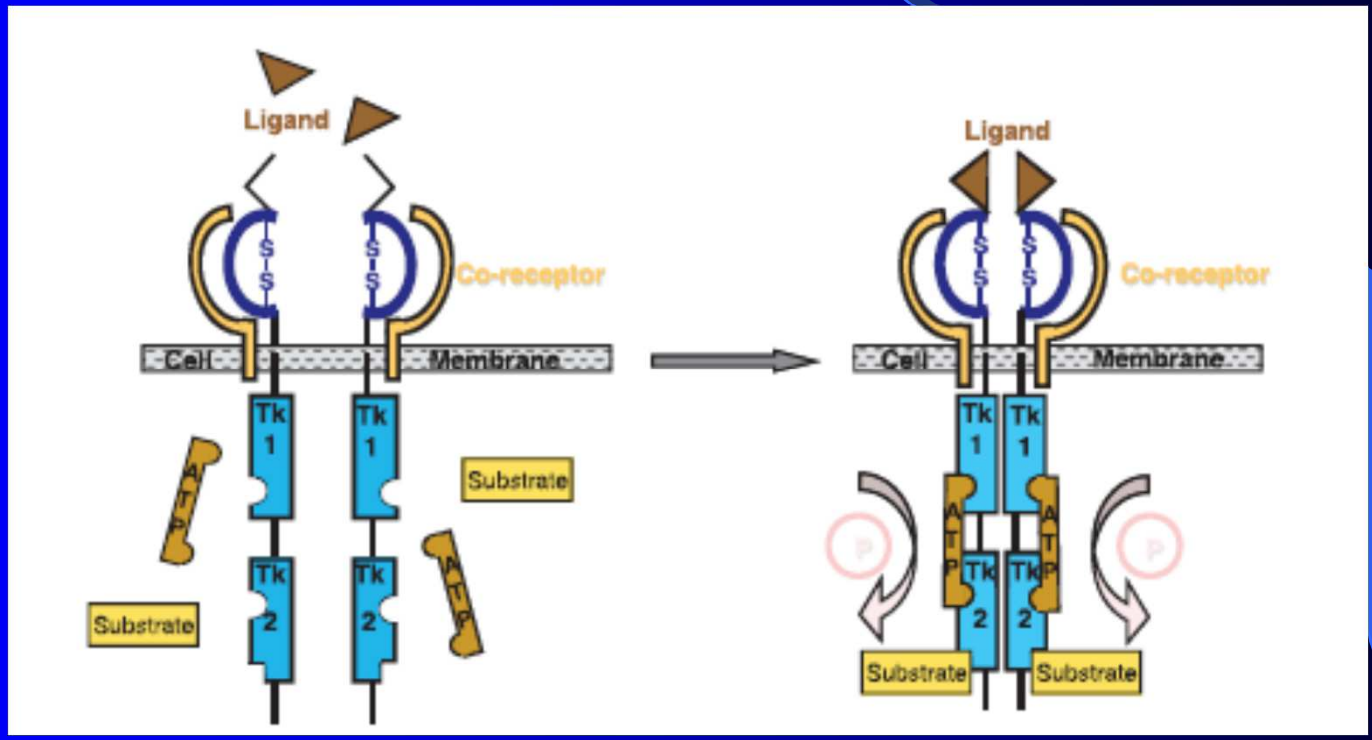
In the presence of GDNF the receptor complex is activated

autofosforilation

signal transduction pathway activation

RET mutations in Endocrinology





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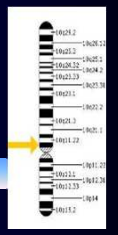
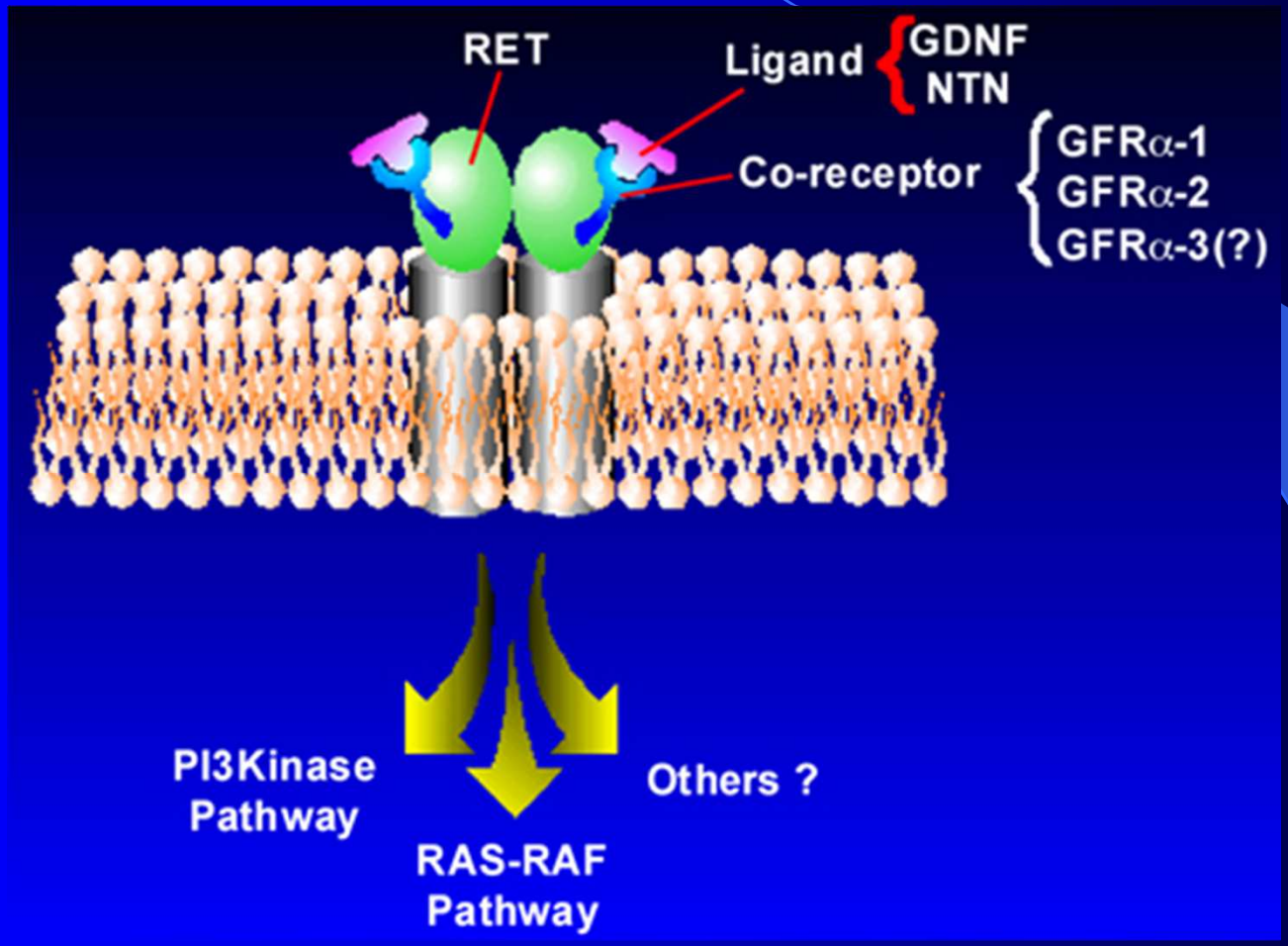
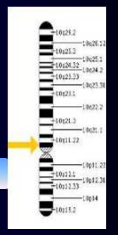
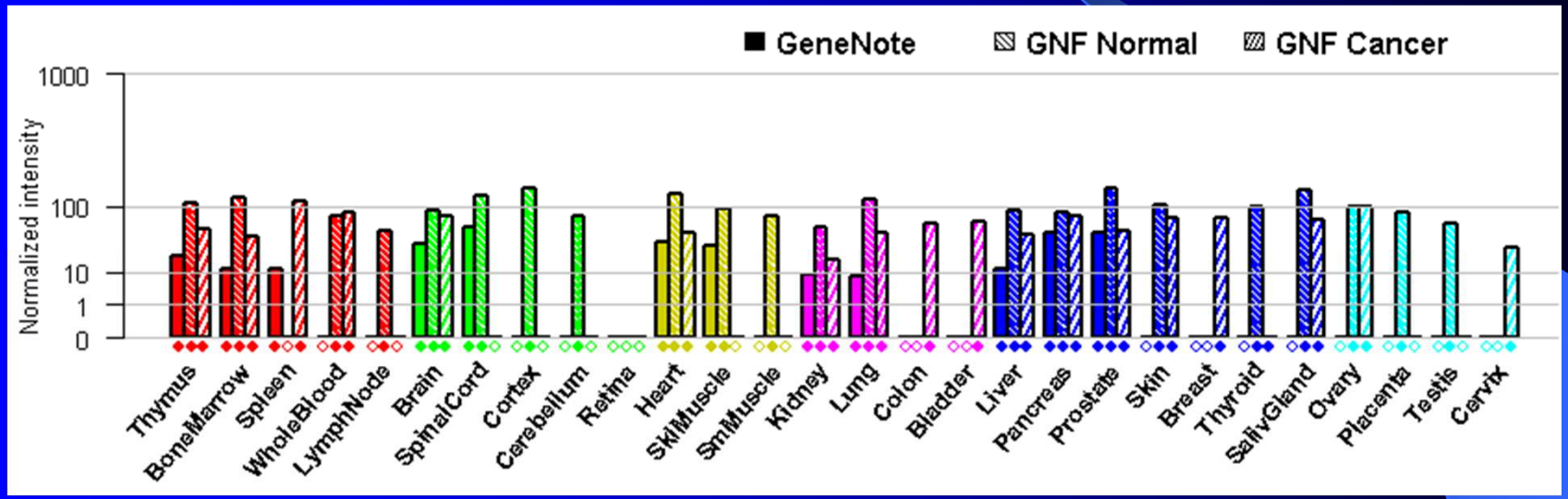




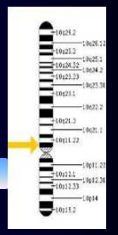
RET mutations in Endocrinology



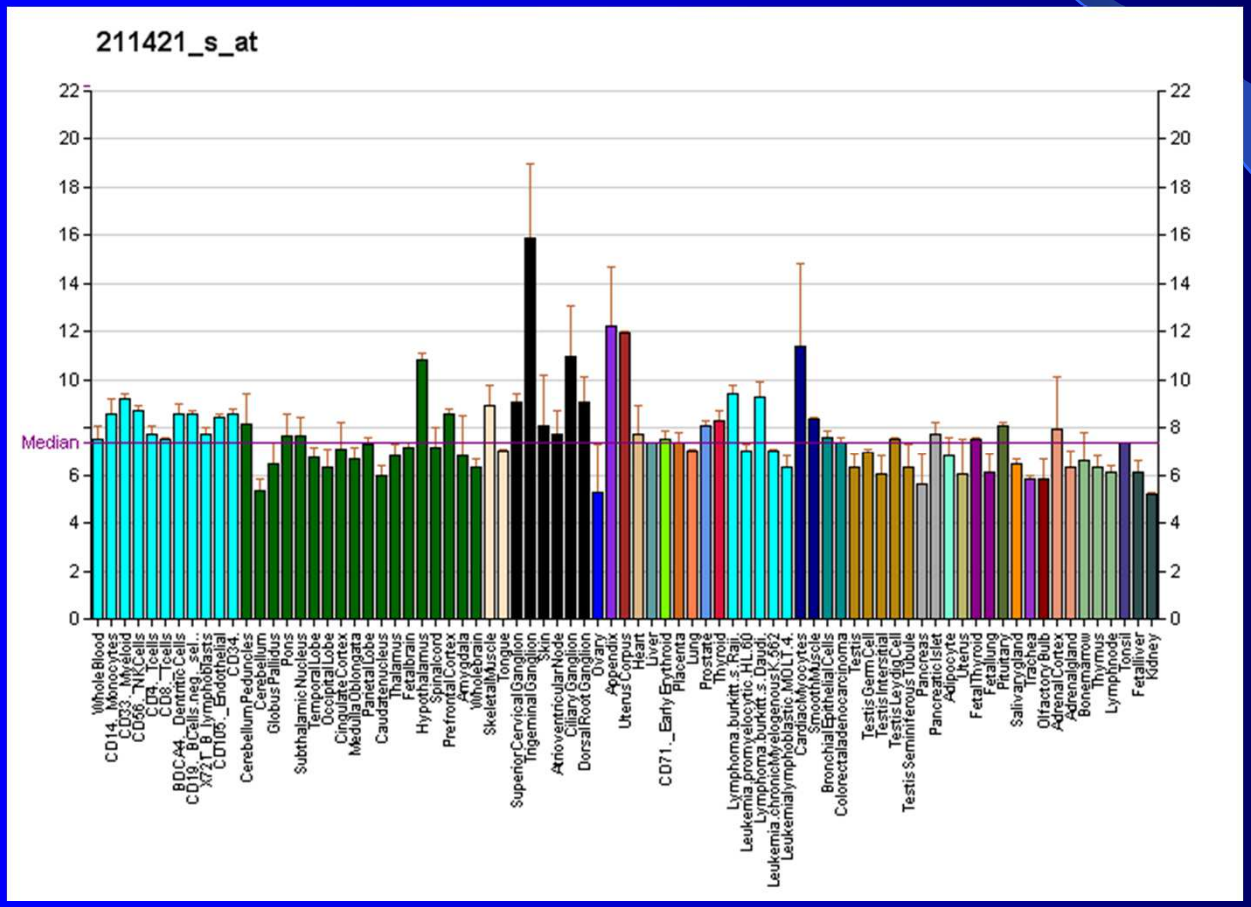
RET is expressed in several normal tissues



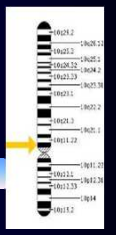
RET mutations in Endocrinology

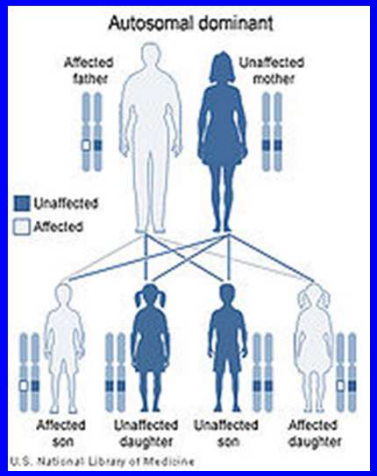


RET is expressed in several normal and neoplastic tissues



RET mutations in Endocrinology





RET mutations

constitutive supraphysiological activation of the RET receptor tyrosine kinase

cell hyperstimulation

thyroid C cells

adrenal medullary cells

parathyroid chief cells

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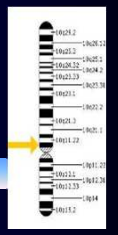
RET mutations in Endocrinology



'Gain-of-function' mutations

transmembrane domain
codon 649

RET protein monomers are kept in close proximity to each other through noncovalent receptor-receptor interactions

Least high transforming ability

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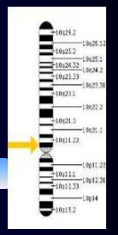
RET mutations in Endocrinology



'Gain-of-function' mutations

cysteine-rich extracellular domains
codons 515, 609, 611, 618, 620, 630, 634

High transforming ability

ligand-independent dimerization and cross-phosphorylation of mutant RET receptor proteins

loss of a cysteine residue irrespective of the amino acid substituting for cysteine

addition of one more cysteine residue in codons 533, 606 or 631

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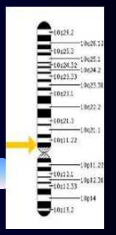
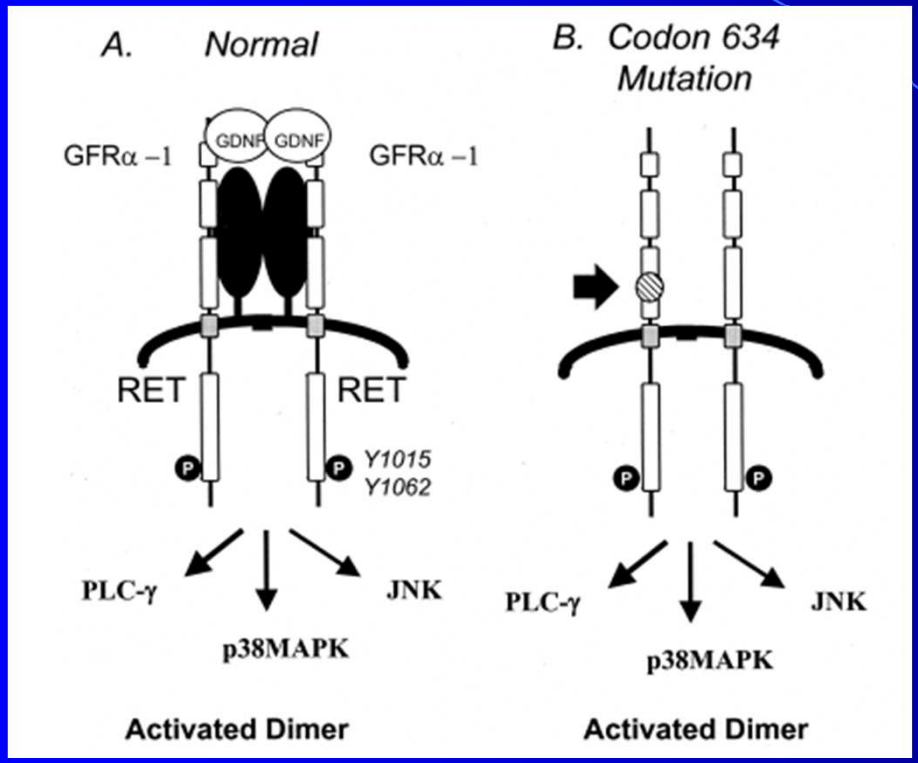
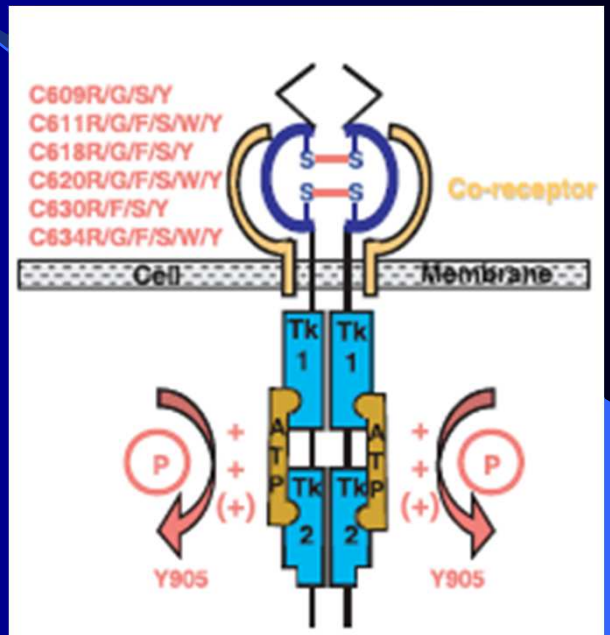
RET mutations in Endocrinology

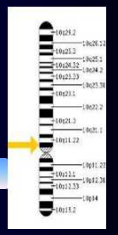






Mutations in the Cystein-rich extracellular domain cause RET dimerization and autophosphorylation, independently of the ligand

RET mutations in Endocrinology



'Gain-of-function' mutations

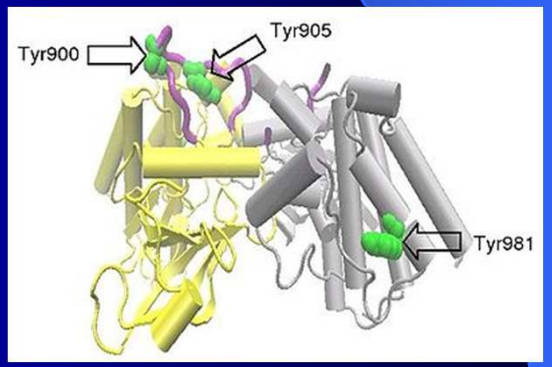
intracellular tyrosine kinase domain
codons 768, 790, 791, 804 and 891

→ facilitate the access of ATP to its binding site

Very high transforming ability

→ preferential binding of intracellular substrate

intracellular catalytic core
codon 918



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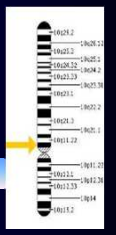
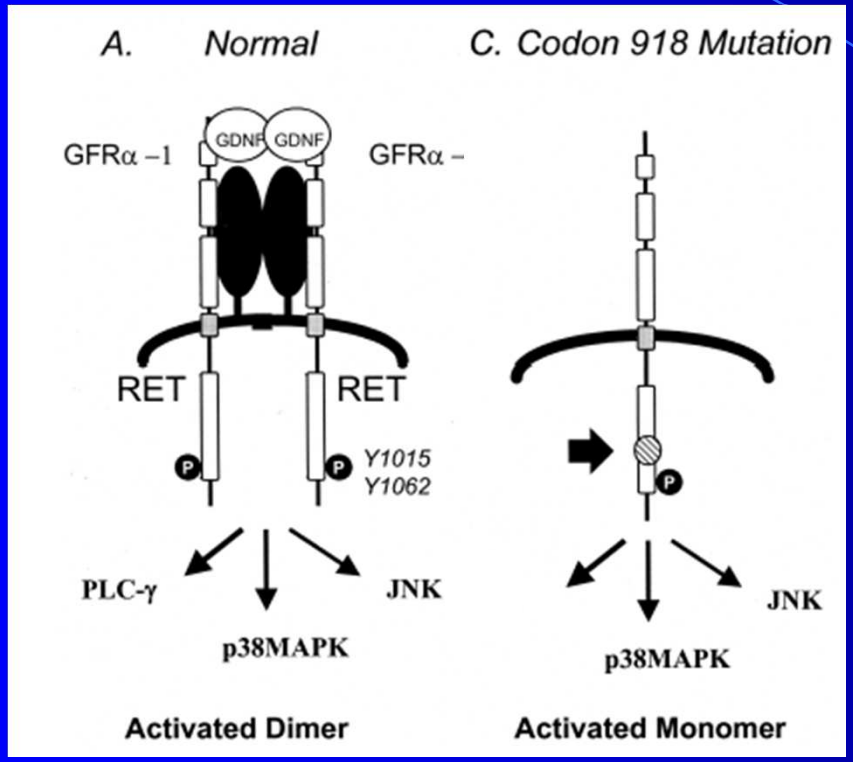
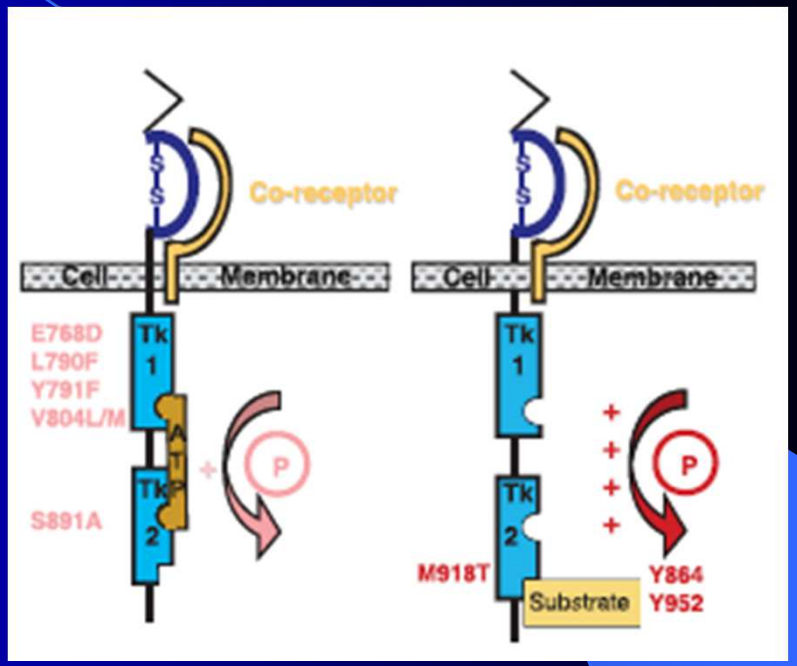
RET mutations in Endocrinology

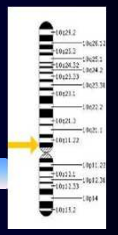


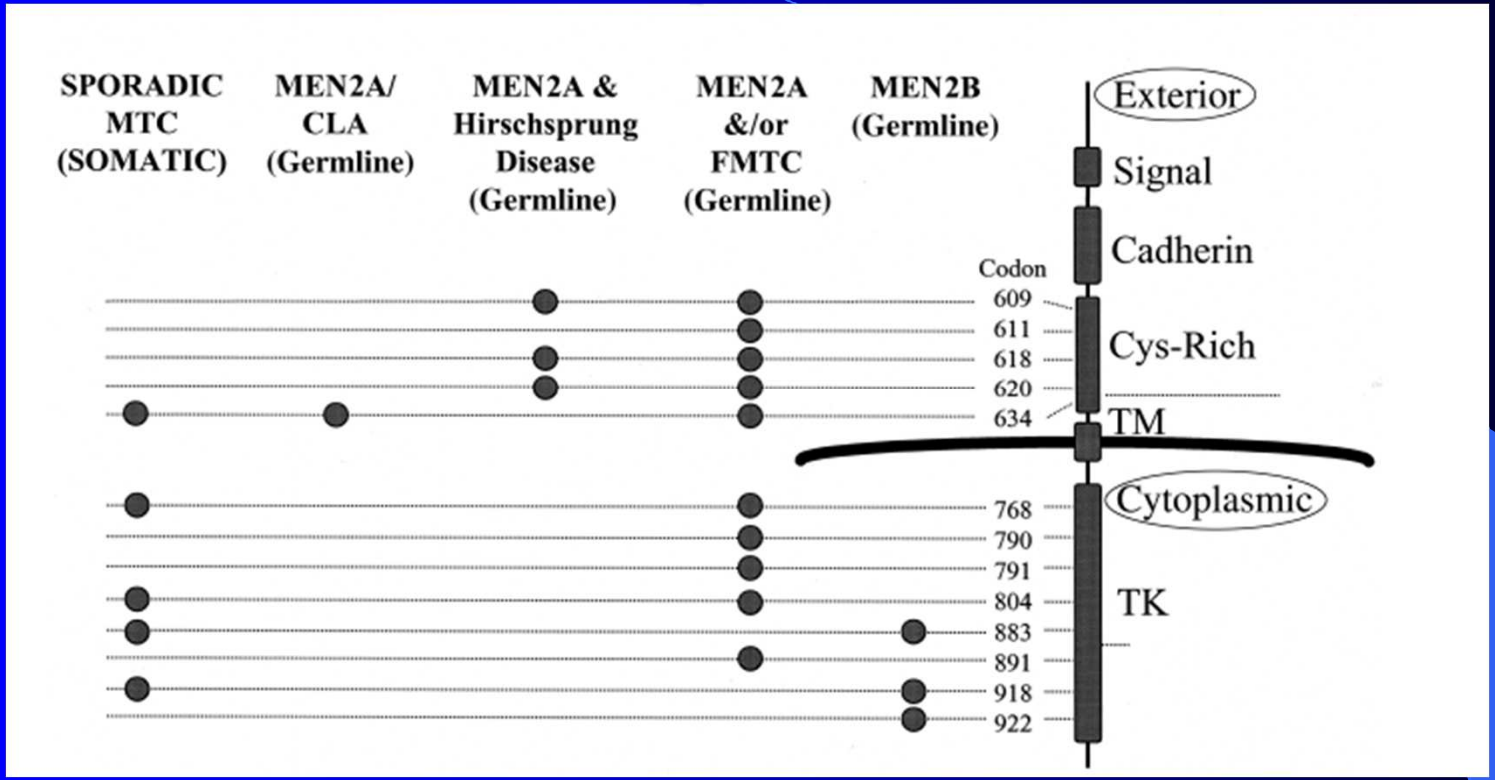




Mutations in the intracellular kinase domain cause RET autophosphorylation, independently of dimerization and of the ligand

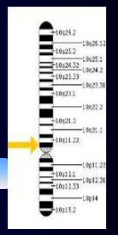
RET mutations in Endocrinology





GENOTYPE-PHENOTYPE CORRELATION

RET mutations in Endocrinology



MEN2A: MTC, hyperparathyroidism, pheochromocytoma

(100% penetrance)

MEN2B: MTC, pheochromocytoma, muco-cutaneous neuromas

(98% penetrance)

FMTC: MTC

Table 36–4. Germline Mutations of the *RET* Proto-oncogene in Multiple Endocrine Neoplasia Type 2*

Affected Codon	Exon	Amino Acid Change Normal→Mutant	Nucleotide Change Normal→Mutant	Clinical Syndrome	Percentage of All MEN2 Mutations
609	10	Cys→Arg	TGC→CGC	MEN2A/FMTC	0–1
		Cys→Tyr	TGC→TAC†		
611	10	Cys→Tyr	TGC→TAC	MEN2A/FMTC	2–3
		Cys→Trp	TGC→TGG	FMTC	
		Cys→Gly	TGC→GGC		
618	10	Cys→Ser	TGC→AGC‡	MEN2A/FMTC	3–5
		Cys→Gly	TGC→GGC		
		Cys→Arg	TGC→CGC		
		Cys→Phe	TGC→TTC		
		Cys→Ser	TGC→TCC		
		Cys→Tyr	TGC→TAC		
		Cys→End	TGC→TGA		
620	10	Cys→Arg	TGC→CGC§	MEN2A/FMTC	6–8
		Cys→Tyr	TGC→TAC‖		
		Cys→Phe	TGC→TTC		
		Cys→Ser	TGC→TCC		
		Cys→Gly	TGC→GGC		
630	11	Cys→Phe	TGC→TTC	FMTC	<0.1
634	11	Cys→Ser	TGC→AGC	MEN2A¶	80–90
		Cys→Gly	TGC→GGC		
		Cys→Arg	TGC→CGC		
		Cys→Tyr	TGC→TAC		
		Cys→Phe	TGC→TTC		
		Cys→Ser	TGC→TCC		
		Cys→Trp	TGC→TGG		
768	13	Glu→Asp	GAG→GAC	FMTC	0–1
790	13	Leu→Phe	TTG→TTT	MEN2A/FMTC	<0.1
791	13	Tyr→Phe	TAT→TTT	FMTC	<0.1
804	14	Val→Met	GTG→ATG	FMTC	0–1
		Val→Leu	GTG→TTG		
883	15	Ala→Phe	GCT→TTT	MEN2B	—
891	15	Ser→Ala	TCG→GCG	FMTC	0–1
918	16	Met→Thr	ATG→ACG	MEN2B	10–20

RET mutations in Endocrinology

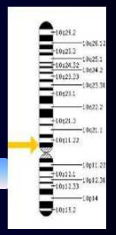


Table 1 Prevalence of RET germline mutations in Continental Europe based on 356 RET families[a]

Risk category	Mutated codon	Germany [64], *n* (%)	France [51, 65], *n* (%)	Italy [66], *n* (%)	Poland [67], *n* (%)	Czech Republic [68], *n* (%)	Total, *n* (%)
++++	918	21 (15)	3 (3)	5 (7)	2 (7)	3 (14)	34 (9.6)
++++	883	0	0	1 (1)	0	0	1 (0.3)
+++	634	57 (40)	46 (47)	23 (33)	9 (33)	11 (50)	146 (41.0)
++(+)	630	1 (1)	0	3 (4)	0	0	4 (1.1)
+	631	1 (1)	0	0	0	0	1 (0.3)
++	620	10 (7)	12 (12)	4 (6)	4 (15)	0	30 (8.4)
++	618	7 (5)	6 (6)	7 (10)	2 (7)	0	22 (6.2)
++	611	2 (1)	1 (1)	1 (1)	0	1 (5)	5 (1.4)
++	609	1 (1)	1 (1)	1 (1)	3 (11)	1 (5)	7 (2.0)
+	533	0	0	0	0	0	0
+	768	2 (1)	2 (2)	3 (4)	0	1 (5)	8 (2.2)
+	790	17 (12)	4 (4)	1 (1)	0	0	22 (6.2)
+	791	10 (7)	0	0	5 (19)	2 (9)	17 (4.8)
+	804	9 (6)	15 (15)	14 (20)	1 (4)	3 (14)	42 (11.8)
+	891	3 (2)	7 (7)	6 (9)	1 (4)	0	17 (4.8)
Total	Any	141	97	69	27	22	356

[a]Considering series with a minimum of 20 RET families only.

Machens et al. 2009 J Intern Med 266: 114

RET mutations in Endocrinology

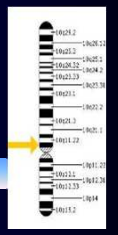
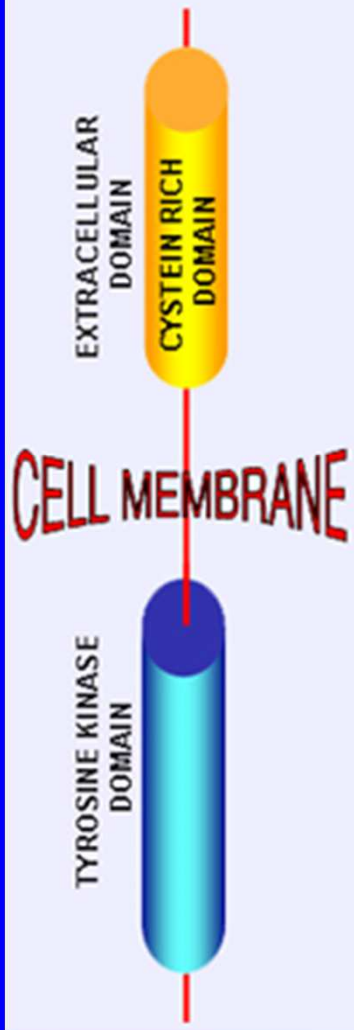


Fig. 2 Genotype-phenotype correlations according to known germline mutations



MUTATED CODONS	EXONS	PHENOTYPE
533	8	FMTC
603	10	FMTC
609		FMTC/MEN 2A
611		FMTC/MEN 2A
618		FMTC/MEN 2A
620		FMTC/MEN 2A
630	11	FMTC
632/633/634		MEN 2A
634		MEN 2A
640		MEN 2A
641		MEN 2A
648		MEN 2A
768	13	FMTC
781		FMTC
790/791		FMTC
804	14	FMTC
844		FMTC
883	15	FMTC/MEN 2B
891		FMTC
904		FMTC/MEN 2B
912	16	MEN 2B
918		MEN 2B
922		MEN 2B

Elisei *et al*. J Clin Endocrinol Metab 2007, 92(12):4725–4729

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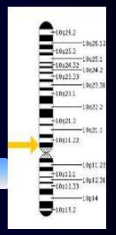


DNA-based screening

Following the discovery of RET mutations in the germline of patients with MEN type 2, DNA-based analysis of the RET proto-oncogene was rapidly integrated into the routine clinical armamentarium.

Machens et al. 2009 J Intern Med 266: 114

Cost-effective identification of affected family members

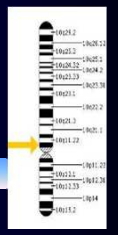
Gilchrist et al. Clin Genet 2004; 66: 349–53

legal and ethical importance indicating the need for prophylactic thyroidectomy

gold standard of care

Rosenthal et al Thyroid 2005; 15: 140–5

RET mutations in Endocrinology



Different forms of medullary thyroid cancer

MTC

75% SPORADIC

5th-6th decade of life

25% HEREDITARY
(INCLUDED IN MULTIPLE ENDOCRINE NEOPLASIA SYNDROMES)

within 3rd decade of life

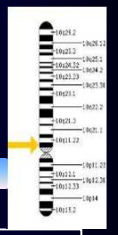
MEN IIA:
- MTC 100%
- PHEOCHROMOCYTOMA 50%
- PARATHYROID MULTPLIE ADENOMATOSIS 30%

MEN IIB:
- MTC 100%
- PHEOCHROMOCYTOMA 45%
- HABITUS MARPHANOID 65%
- MUCOSAL NEURINOMAS 100%
- MEGACOLON 30%
- CORNEAL NERVE HYPERTROPY 50%

FMTC:
- MTC 100% (ALONE)

Elisei R 2008 Best Pract Res Clin Endocrinol Metab 22: 941–953

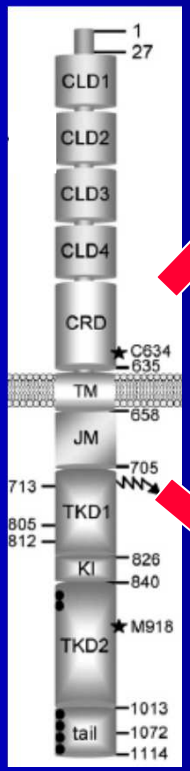
RET mutations in Endocrinology



Hereditary MTC ← Prospective family screening

Germline RET mutations

- 95% of MEN2A kindreds
  - Hirshprung disease
  - Lichen amyloidosis
- 88% of FMTC kindreds
- >95% of MEN 2B kindreds (codon 918)



MUTATED CODONS	EXONS	PHENOTYPE	N C
609	10	FMTC	
611		FMTC	
618		FMTC-MEN 2A	
620		FMTC	
630	11	FMTC	
634		FMTC-MEN 2A	
768	13	FMTC	
790	13	FMTC	
804	14	FMTC	
848	14	FMTC	
883	15	FMTC	
891	15	FMTC	
904	15	FMTC	
918	16	MEN 2B	

Elisei et al. 2007 J Clin Endocrinol Metab 92:4725-9

Castellone et al. 2008 Endocrinol Metab Clin North Am 37:363-74, viii

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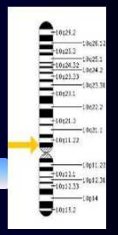
RET mutations in Endocrinology



Hereditary MTC

6-75% of "sporadic" MTC carry a germline RET mutation

Elisei et al. 2007 J Clin Endocrinol Metab 92:4725-9

Wohllk et al. 1996 J Clin Endocrinol Metab 81: 3740-3745

RET genetic testing should be encouraged
in all newly diagnosed MTC patients

The National Comprehensive Cancer Network
Clinical Practice Guidelines in Oncology
2009

biochemical screening
for MEN2

hyperparathyroidims

pheochromocytoma

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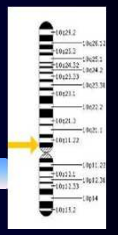
RET mutations in Endocrinology



Genetic screening in proband and in first degree relatives is fundamental

High likelihood of developing MTC during lifespan

consider prophylactic thyroidectomy

follow up

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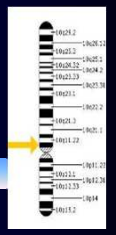
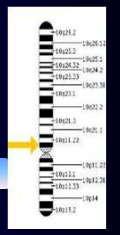
RET mutations in Endocrinology



How is genetic analysis perfomed?

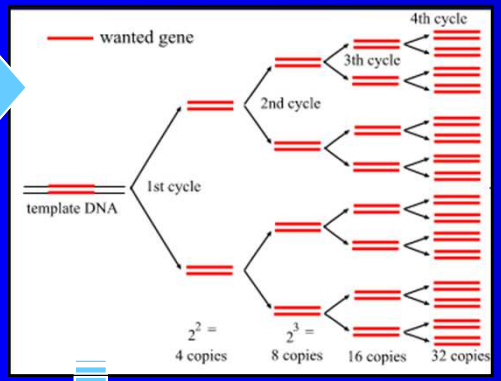
1. Patient referral for MTC or family history of MTC
2. History – evaluate family history
3. Clinical examination
4. Informed consent signature
5. Blood withdrawal (no fasting needed)
6. Sample sent to the Lab

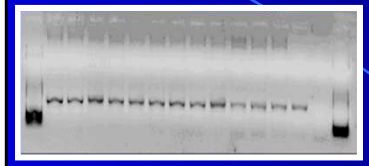
RET mutations in Endocrinology



GENOMIC DNA DIRECT SEQUENCING

DNA isolation

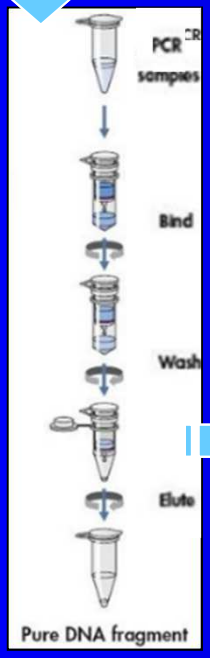




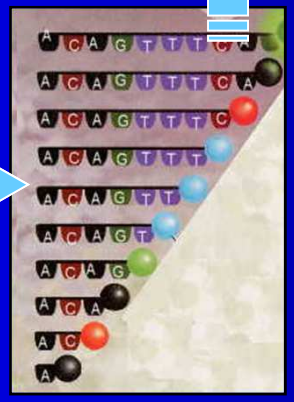
denaturation

Capillary electrophoresis

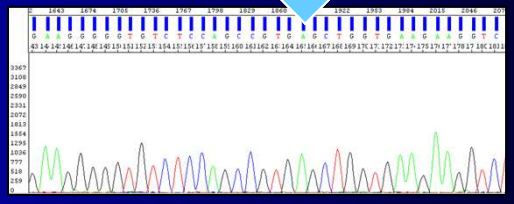
purification



purification



Sequencing reaction



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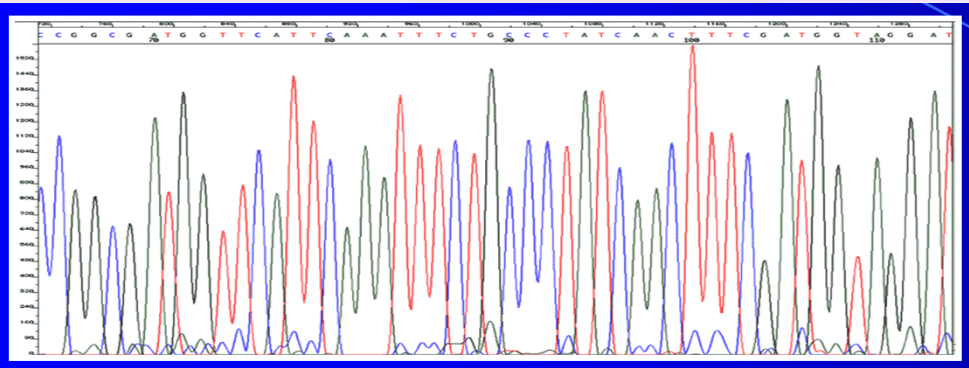
RET mutations in Endocrinology

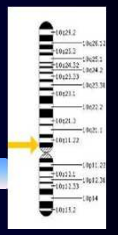


Electropherogram analysis
by a Technicial
by the Physicial in charge

Comparison with the normal sequence
➔ any SNP?

DIAGNOSIS

RET mutations in Endocrinology



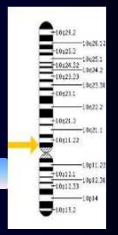
Risk level	Risk	RET genotype (mutation in codon)
3	Highest	883, 918, 922
2	High	634, 630, 609, 611, 618, 620
1	Least high	768, 790, 791, 804, 891

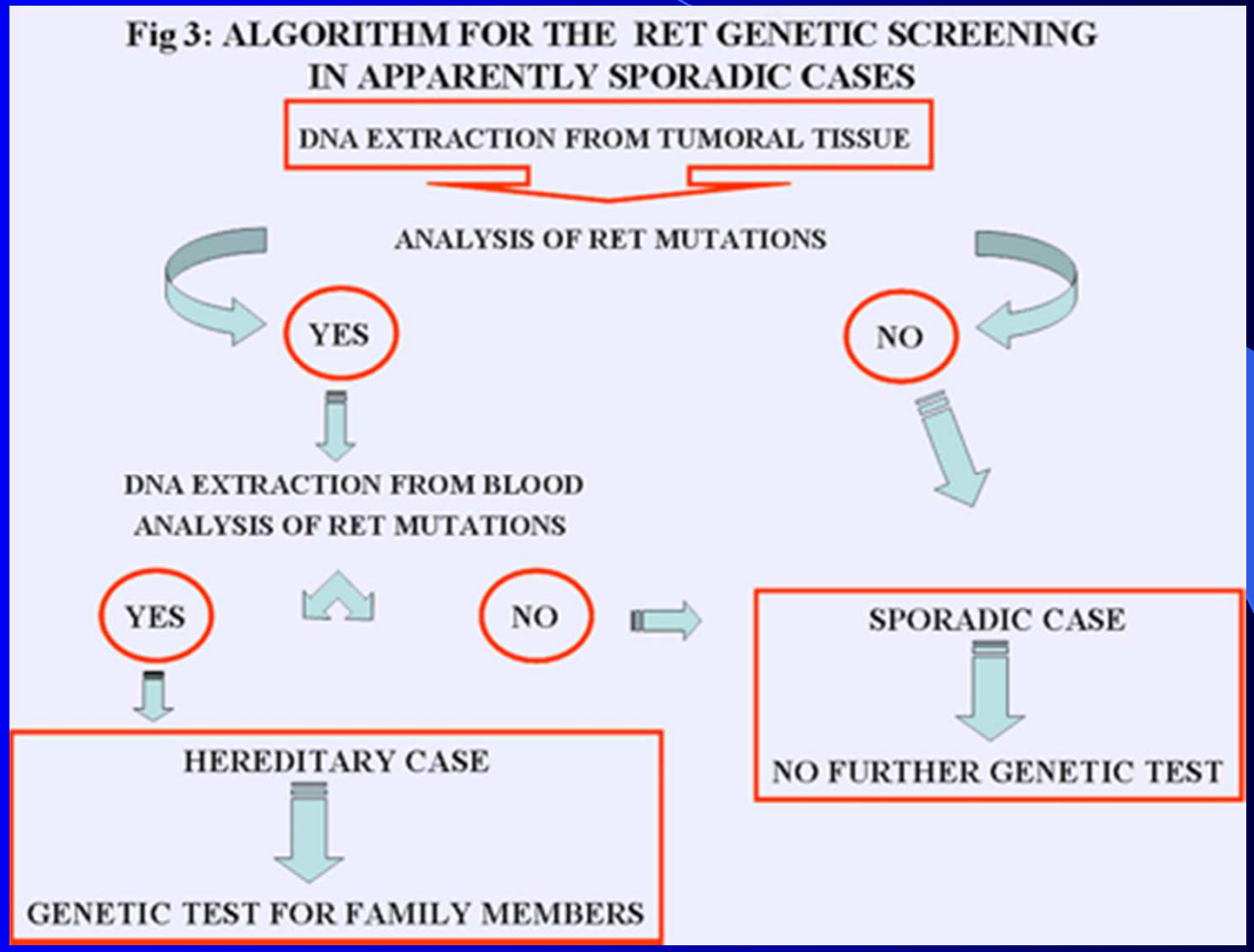
Brandi et al. 2001 J Clin Endocrinol Metab 86: 5658–71

mutation site is one of the most important determining factors of risk and age-related penetrance of a specific RET mutation

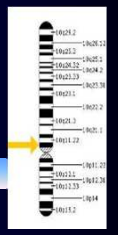
Moore et al. 2008 Pediatr Surg Int 24:521–530

RET mutations in Endocrinology





RET mutations in Endocrinology

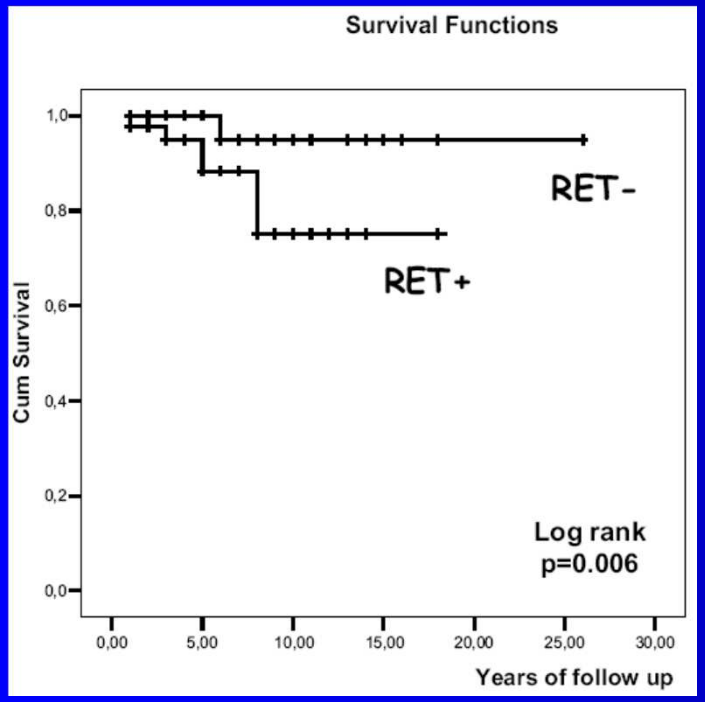


Sporadic MTC

40-50% of sporadic MTC display somatic RET mutations

Romei et al. 1996 J Clin Endocrinol Metab 81:1619–1622
Schilling et al. 2001 Int J Cancer 95:62–66

Zedenius et al. 1995 J Clin Endocrinol Metab 80:3088–3090
Zedenius et al. 1998 Cancer Detect Prev 22:544–548



Somatic RET mutations correlate with

- presence of lymph node metastases at diagnosis
- worse outcome
- disease persistence after surgery
- lower survival rate

bad prognostic factor

Elisei et al. 2008 J Clin Endocrinol Metab 93:682-7

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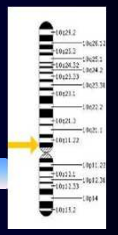
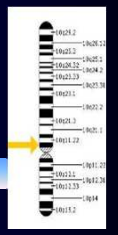
RET mutations in Endocrinology



Tab 1 . Somatic mutations and deletions in sporadic MTC

Amino acid substitution	Nucleotide substitution	Reference
A876V	2627C>T	Uchino et al 1999
A883F	2647_2648GC>TT	Elisei et 2007
A919V	c.2756C>T	Uchino et al 1998
C630R	c.1888T>C	Bugalho et al 1997
C634A	c.?	Romei et al 1996
C634R	c.?	Elisei et 2007
C634T	c.?	Romei et al 1996
C634W	c.1902C>G	Elisei et 2007
C634Y	c.?	Elisei et 2007
D631_L633>E	c.1893_1898delCGAGCT	Musholt et al 1997
D631G	c.1892A>G	Shirahama et al 1998
D898_E901del	c.2694_2705del12	Uchino et al 1999
E632_A640>VRP	c.1895_1918>TGCGGC	Marsh et 1998
E632_C634>L	c.1895_1900delAGCTGT	Kimura et al 1995
E632_L633del	c.1894_1899delGAGCTG	Ceccherini et al 1997
E768D	c.2304G>C	Cho et al 2005
E884K	c.2650G>A	Uchino et al 1999
E901K	c.2701G>A	Uchino et al 1999
E921K	c.2761G>A	Dvoráková et al 2006
F612_C620del	c.1834_1860del27	Kalinin et al 1998
G592_G607del	c.1774_1821del48	Ceccherini et al 1997
G748C	c.2242G>T	Uchino et al 1999
G911D	c.2732G>A	Dvoráková et al 2006
M918T	c.2753T>C	Dvoráková et al 2006
P766S	c.2296C>T	Bugalho et al 1997
R908K	c.2723G>A	Uchino et al 1999
V591I	c.1771G>A	Dvoráková et al 2006
V778V	c.2334C>T	Uchino et al 1998

RET mutations in Endocrinology



SURGERY

total thyroidectomy with dissection of ipsilateral and central neck compartments

? contralateral dissection ?

Sporadic MTC

Hereditary MTC

Asymptomatic RET mutation carriers before MTC occurrence

↳ undetectable CT levels in > 95% of cases

Kouvaraki et al. Thyroid 2005 15: 531–544

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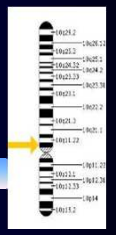
RET mutations in Endocrinology



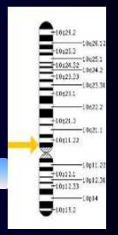
SURGERY

Ideally, prophylactic thyroidectomy should be undertaken before MTC develops in children

- normal thyroid volume
- asymptomatic
- young

Miccoli et al. J Endocrinol Invest 2004;27: 557–61
Miccoli et al. Surg Endosc 2007;21: 120–3

RET mutations in Endocrinology



SURGERY

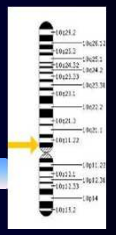
Hereditary MTC

	Risk level	Risk	Surgery within
codon 918	3	Highest	within the first 6 months of life
codons 609, 611, 618, 620, 630, 634	2	High	before 5 years of age
codons 768, 790, 791, 804 and 891	1	Least high	between 5 and 10 years of age

Falchetti et al. 2008 Best Pract Res Clin Rheumatol 22:149-63

aggressive neck dissection should be performed with lateral lymph node involvement

RET mutations in Endocrinology



SURGERY

Very early surgical intervention (<2 yr for exon 9187 mutations or <10 yr)

no need for additional lymph node dissection

Preserve parathyroid glands → autograft

≥ 10 involved lymph nodes → no surgical cure

Machens et al. Cancer 2000; 88: 1909–15.
Weber et al.. Surgery 2001; 130: 1044–9.
Scollo et al. J Clin Endocrinol Metab 2003; 88:2070–5.

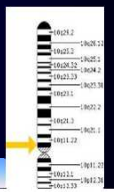
neck lymph node metastases dissection → avoid reoperations

Thomusch et al. Surgery 2003; 133: 180–5.
Dralle et al. Surgery 2004; 136: 1310–22.

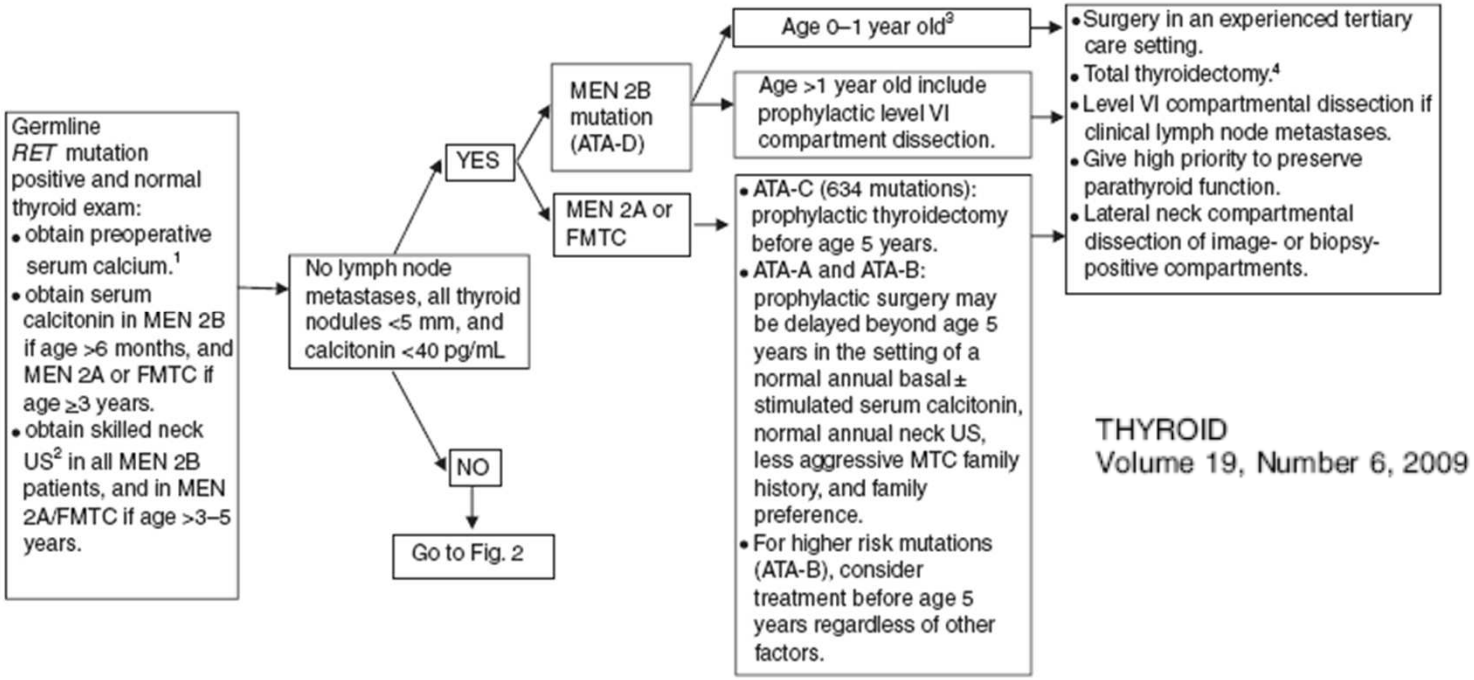
neck lymph node metastases dissection → eliminate the possibility of extranodal growth

Machens et al. Surgery 2001; 129: 23–8

RET mutations in Endocrinology



Medullary Thyroid Cancer: Management Guidelines of the American Thyroid Association



THYROID
Volume 19, Number 6, 2009

[1]Treat hyperparathyroidism with 4 gland resection and autograft to heterotopic site, or subtotal parathyroidectomy. Consider cryopreservation. PHEO preoperative screening should begin by age 8 years for MEN 2B and mutated *RET* codons 634 and 630; otherwise by age 20 years for other *RET* mutations.

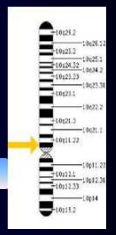
[2]Neck US to include the superior mediastinum and central and lateral neck compartments.

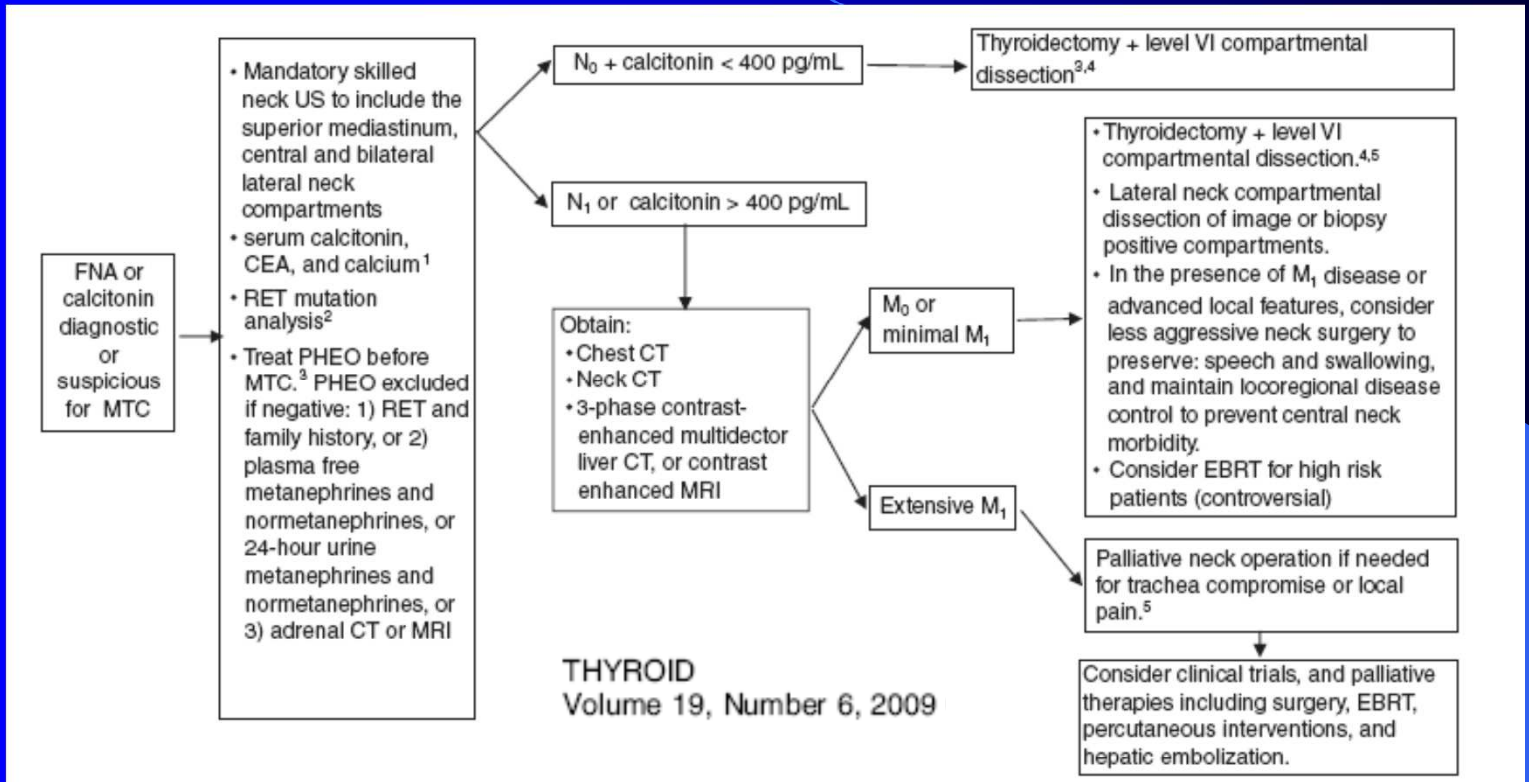
[3]Insufficient data to recommend routine prophylactic level VI compartment dissection.

[4]Parathyroid glands resected or devascularized should be autografted in the neck in *RET*-negative, MEN 2B, and FMTC patients, while MEN 2A glands should be auto graphed to a heterotopic site.

FIG. 1. Initial diagnosis and therapy of pre-clinical disease.

RET mutations in Endocrinology





[1]Treat hyperparathyroidism with 4 gland resection and autograft to heterotopic site, or subtotal parathyroidectomy. Consider cryopreservation.

[2]Ideally performed with genetics counseling and completed preoperatively.

[3]PHEO preoperative screening should begin by age 8 years for MEN 2B and mutated *RET* codons 634 and 630; and by age 20 years for other RET mutations.

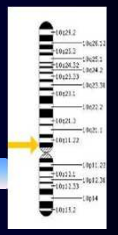
[4]Parathyroid glands resected or devascularized should be autografted in the neck in *RET*-negative, MEN 2B, and FMTC patients, while MEN 2A glands should be autografted to a heterotopic site.

[5]Consider external beam radiation of TNM stage T4 disease to prevent recurrent local disease.

FNA, fine-needle aspiration biopsy.

FIG. 2. Initial diagnosis and therapy of clinically apparent disease.

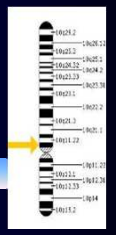
RET mutations in Endocrinology



TYROSINE KINASE INHIBITORS

Compound	Generic name	Structure	Targets[a]	Reference
ZD6474 (AstraZeneca, Wilmington, DE)	Vandetanib	Anilinoquinazoline	RET (130 nmol/l) VEGFR2 (40 nmol/l) VEGFR3 (110 nmol/l) EGFR (500 nmol/l)	Herbst *et al.*[39]
BAY 43-9006 (Bayer Pharmaceuticals, Leverkusen, Germany)	Sorafenib	Bis-aryl-urea	RET (47 nmol/l) VEGFR1 (26 nmol/l) VEGFR2 (90 nmol/l) VEGFR3 (20 nmol/l) PDGFRβ (57 nmol/l) FLT3 (33 nmol/l) KIT (68 nmol/l) FGFR1 (580 nmol/l) RAF (6 nmol/l) BRAF (25 nmol/l) p38 MAPK (38 nmol/l)	Wilhelm *et al.*[44]
AMG 706 (Amgen, Thousand Oaks, CA)	Motesanib	Nicotinamide	RET (59 nmol/l) VEGFR1 (2 nmol/l) VEGFR2 (3 nmol/l) VEGFR3 (6 nmol/l) PDGFRβ (84 nmol/l) FLT3 (33 nmol/l) KIT (8 nmol/l)	Polverino *et al.*[47]
SU011248 (Pfizer, New York, NY)	Sunitinib	Indolin-2-one	RET (100 nmol/l) VEGFR2 (4 nmol/l) PDGFRβ (39 nmol/l) FLT3 (8–14 nmol/l) KIT (1–10 nmol/l) FGFR1 (880 nmol/l) CSF1R (50–100 nmol/l)	Chow and Eckhardt[46]

RET mutations in Endocrinology



TYROSINE KINASE INHIBITORS

phase II trials

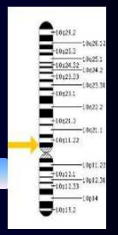
- prolonged disease stabilization
- clinical benefits in 50% of the patients
- partial tumor responses in only few patients
- no improvement in survival so far

Sherman J Clin Endocrin Metab published online March 3, 2009

targeting RET is not sufficient to treat all MTC tumors

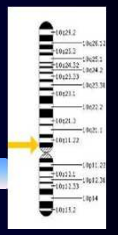
Schlumberger et al. Nat Clin Pract Endocrinol Metab. 2008;4:22-32

RET mutations in Endocrinology



- Most MTC cases are cured by initial surgery when performed at an early stage
- Disease can persist or recur and metastasis can occur, with potentially serious effects on quality of life and mortality

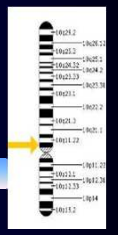
RET mutations in Endocrinology



- New drugs that rely on knowledge of molecular oncology in MTC are available and are needed for patients with metastatic MTC

- Well-designed clinical trials should help in selecting the most active compounds

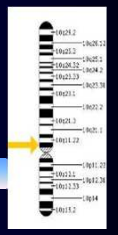
RET mutations in Endocrinology

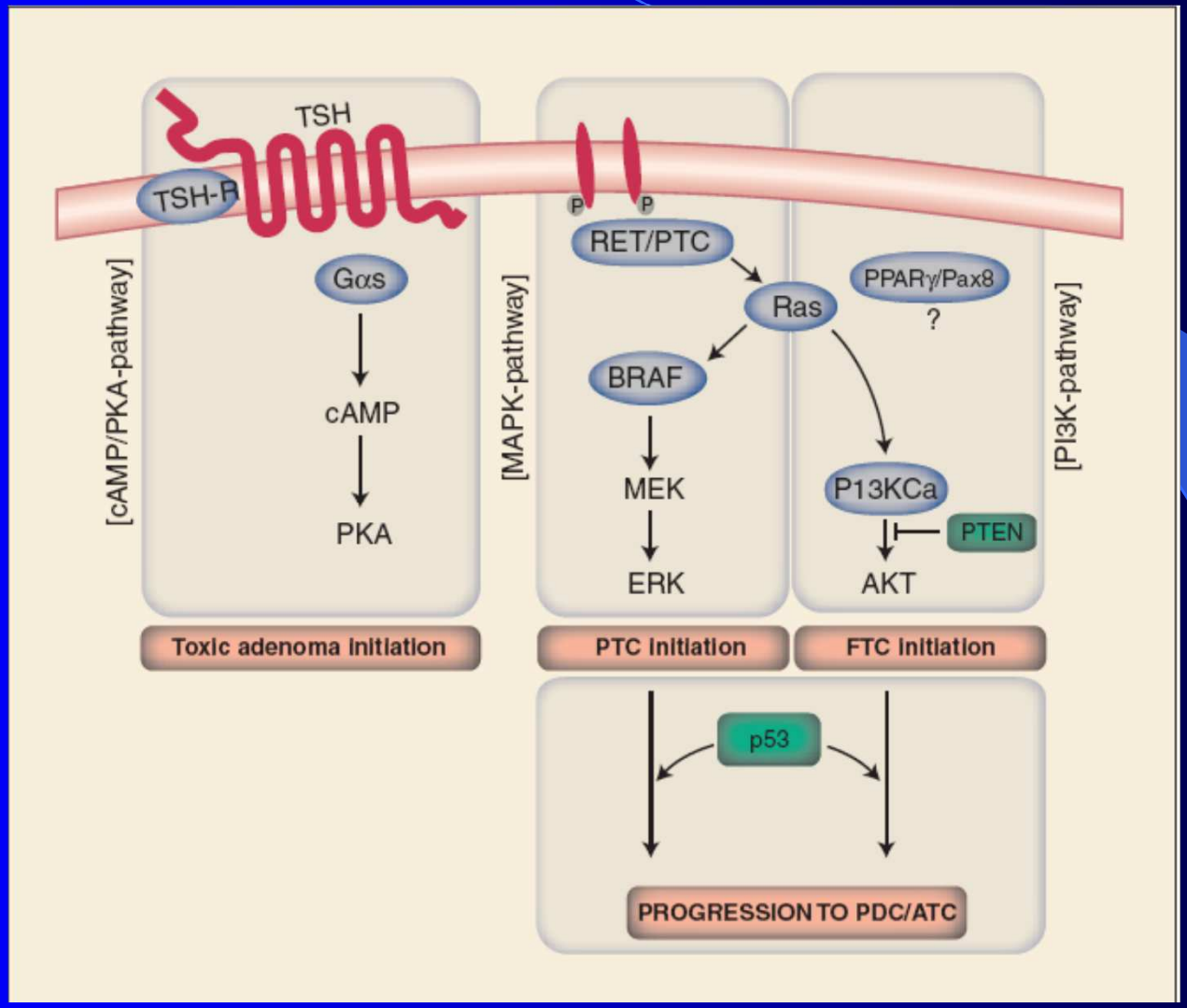


RET mutation analysis is a fundamental step in the diagnostic work-up in medullary thyroid carcinoma patients

Nuove acquisizioni sulla patogenesi dei tumori tiroidei

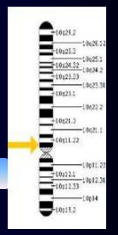
THYROID CANCER PATHOGENESIS





Riesco-Eizaguirre et al. Clin Transl Oncol 2007, 9:686-693

THYROID CANCER PATHOGENESIS



Average prevalence of mutations in thyroid cancer

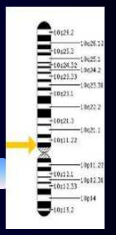
Tumor type	Prevalence (%)
Papillary carcinoma	
BRAF	45
RET/PTC	20
RAS	10
TRK	<5
Follicular carcinoma	
RAS	45
PAX8-PPARg	35
PIK3CA	<10
PTEN	<10

Tumor type	Prevalence (%)
Medullary carcinoma	
Familial forms of RET	>95
Sporadic RET 50	
Poorly differentiated carcinoma	
RAS	35
β-Catenin (CTNNB1)	20
TP53	20
BRAF	15
Anaplastic carcinoma	
TP53	70
β-Catenin (CTNNB1)	65
RAS	55
BRAF	20

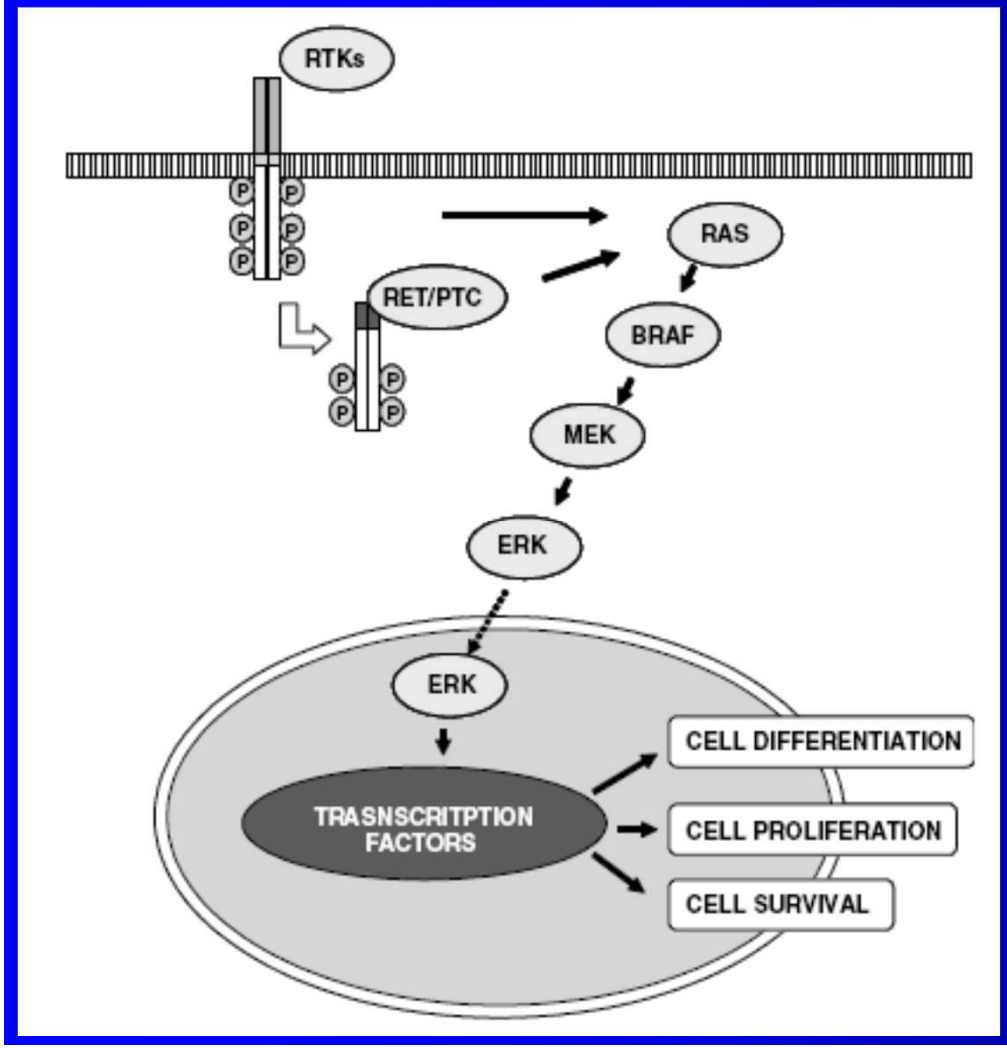
Nikiforov Y. Modern Pathology 2008, 21: S37–S43

EFE 2009

THYROID CANCER PATHOGENESIS



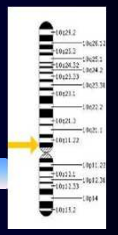
MAPK signaling pathway



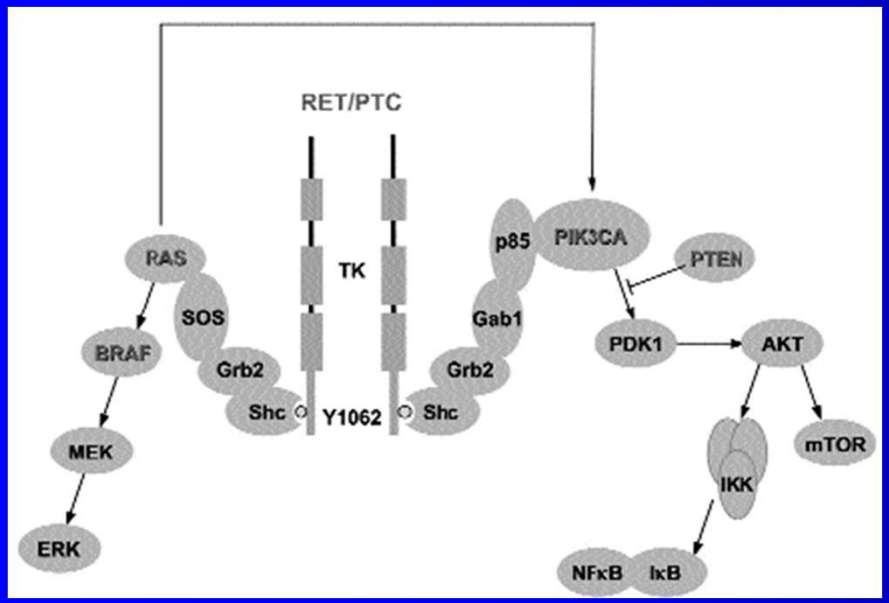
Alterations can occur at different levels in thyroid cancer

Nikiforov Y. Modern Pathology 2008, 21: S37–S43

THYROID CANCER PATHOGENESIS



RET/PTC rearrangement



intact RET TK domain

↳ binding to SHC

↳ RAS-RAF-MAPK activation

RET ➔ cell membrane receptor tyrosine kinase

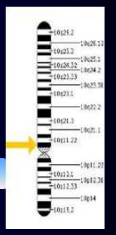
activated by chromosomal rearrangement

RET 3' portion fused to various unrelated genes 5' portion

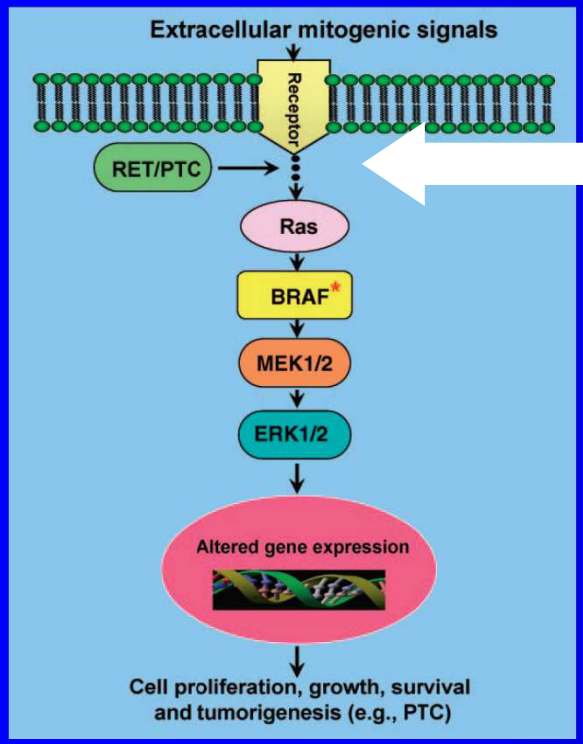
RET/PTC1 = RET + H4

RET/PTC3 = RET + NCOA4

THYROID CANCER PATHOGENESIS



PAPILLARY CARCINOMA

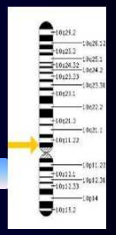


RET/PTC1 and RET/PTC3 paracentric inversions

- ✓ 20% of PTC, mainly classical histology
- ✓ younger age
- ✓ radiation exposure
- ✓ ↑ lymph node metastases
- ✓ lower stage (micro)
- ✓ found in adenomas and benign lesions

Nikiforova et al. Exp Rev Mol Diagn 2008, 8: 83

THYROID CANCER PATHOGENESIS



RET/PTC rearrangement

transforms thyroid cells in culture

Santoro et al. Cell Growth Differ 1993;4:77–84

gives rise to thyroid carcinomas in transgenic mice

Jhiang et al. Endocrinology 1996;137:375–378
Santoro et al. Oncogene 1996;12:1821–1826
Powell et al. Cancer Res 1998;58:5523–5528

requires a functional BRAF signaling

Melillo et al. J Clin Invest 2005;115:1068–1081

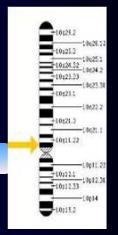
inhibits thyroid-specific gene expression

increases cell proliferation

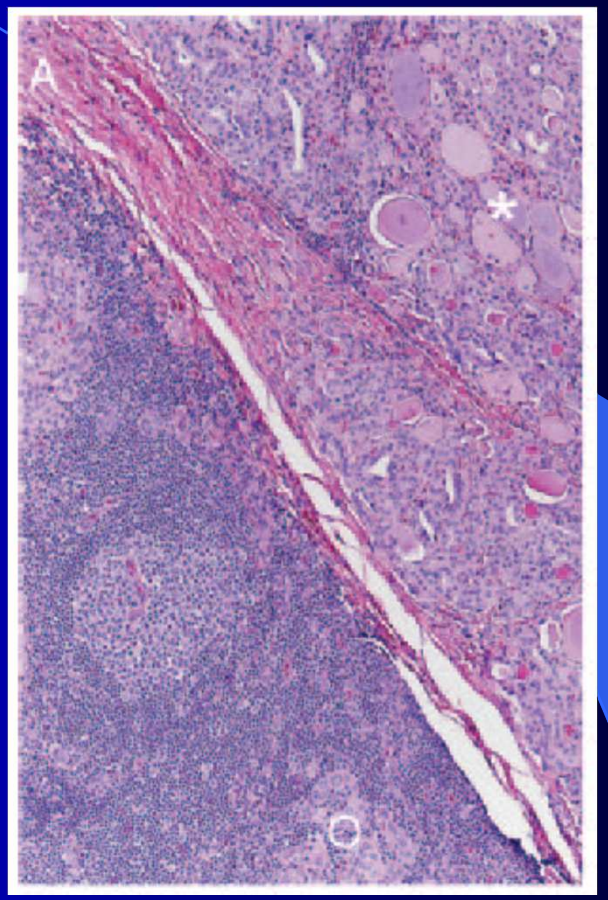
Mitsutake et al. Cancer Res 2005;65: 2465–2473

tumorigenesis

THYROID CANCER PATHOGENESIS



RET/PTC rearrangement



Found in 62% of HT

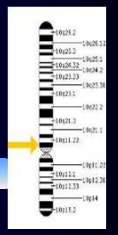
Sheils OM et al. Int J Surg Pathol 2000 ,8:185–189
Wirtschafter A et al. Laryngoscope 1997, 107:95–100
Rhoden KJ et al. J Clin Endocrinol Metab 2006, 91: 2414–2423

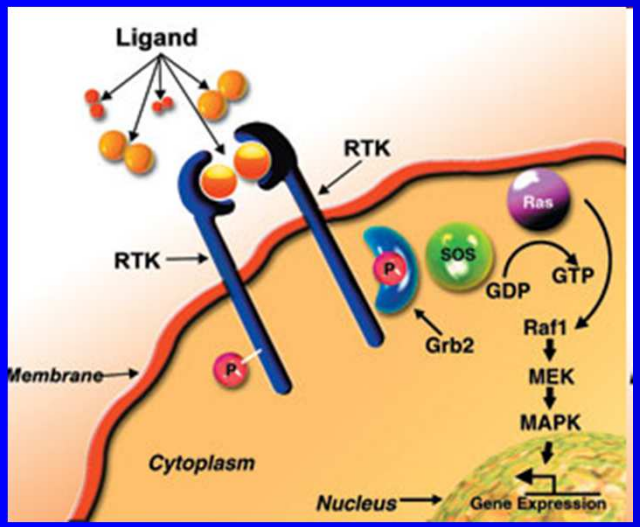
occult neoplasm ?

Bias!

technical limitations
high false positive results
lack of reproducibility

THYROID CANCER PATHOGENESIS





RAS mutations

H-RAS, K-RAS, N-RAS

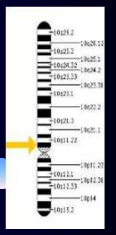
- highly related G-proteins located at the inner surface of the cell membrane
- mediate intracellular signal transduction from cell membrane receptors

many human neoplasms display point mutations

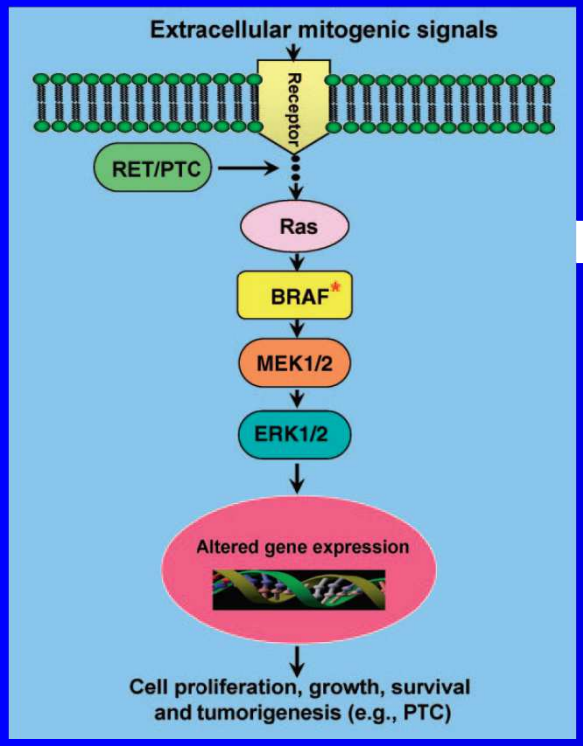
mutant protein

permanently in active conformation
constitutively activates signaling pathways

THYROID CANCER PATHOGENESIS



PAPILLARY CARCINOMA



NRAS, HRAS or KRAS point mutations

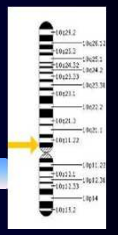
- ✓ 15-20% of PTC, mainly follicular variant
- ✓ ↑encapsulation
- ✓ ↓↑ lymph node metastases

Adeniran et al. Am J Surg Pathol 2006, 30: 216
Hara et al. Surgery 1994;116:1010–1016

- ✓ also found in benign lesions

Nikiforova et al. Exp Rev Mol Diagn 2008, 8: 83

THYROID CANCER PATHOGENESIS



PAPILLARY CARCINOMA

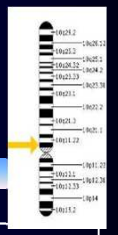
RAS mutations

- (+) → TSH-independent growth
- (+) → TSH-dependent apoptosis
- (+) → de-differentiation
- (+) → DNA damage

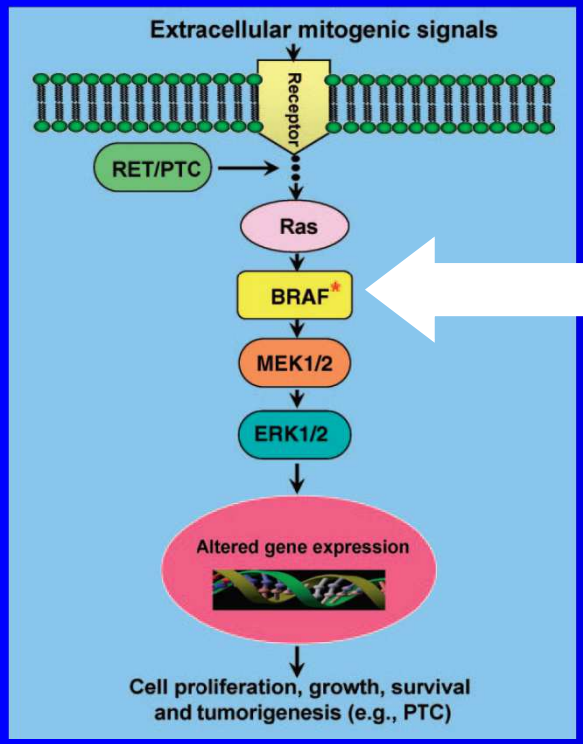
→ genomic instability

Riesco-Eizaguirre et al. Endocrine-Related Cancer 2007, 14:957

THYROID CANCER PATHOGENESIS



PAPILLARY CARCINOMA



BRAF V600E point mutation

[K601E and V599Ins]

- ✓ 45-80% of PTC, mainly tall cell and classic hystology
- ✓ ↑ extrathyroidal invasion
- ✓ higher stage
- ✓ ↑ recurrence (with reduced I up-take)

Lupi et al. J Clin Endocrinol Metab. 2007;92:4085

- ✓ ↑ de-differentiation

resticted to PTC

Nikiforova et al. Exp Rev Mol Diagn 2008, 8: 83

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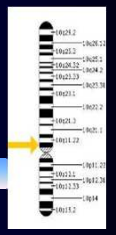
THYROID CANCER PATHOGENESIS



PAPILLARY CARCINOMA

BRAF mutation(s)

BRAF up-regulates genes related to thyroid inflammation

Melillo et al. J Clin. Invest 2005, 115: 1068–1081

(−) → NIS expression

(−) → NIS trafficking to the membrane

Riesco-Eizaguirre et al. Endocrine-Related Cancer 2006, 13: 257

(+) → DNA synthesis and apoptosis

→ little growth advantage

BUT

genomic instability

Mitsutake et al. Cancer Research 2005;65: 2465

(+) → MMP, vimentin, osteopontin

epithelial-mesenchimal transition

Vasko et al. Curr Opin Oncol 2007;19: 11

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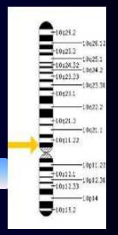
THYROID CANCER PATHOGENESIS



BRAF mutation screening

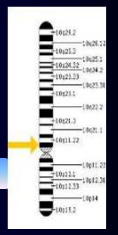
Helpful

- to evaluate surgical aggressiveness of neck dissection
  - to reduce PTC recurrence rate and avoid increase in complications

- to plan aggressiveness of medical management

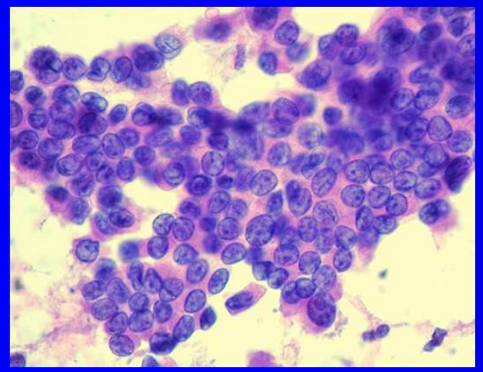
Xing M Endocr Rev 2007,28:742

THYROID CANCER PATHOGENESIS



High accuracy of BRAF mutation detection for PTC on FNAB specimens

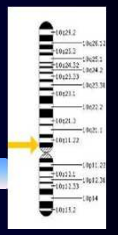
Xing M Endocr Relat Cancer 2005,12:245



cytologically diagnosed PTC

preoperative BRAF mutation test on FNAB?

THYROID CANCER PATHOGENESIS



BRAF mutation

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University of Ferrara

diagnostic FNAB from October 2007 to December 2008

suspicious for malignancy according to AACE/AME guidelines

Gharib et al. Endocr Pract 2006 **12** 63-102.

Solid isoechoic nodules
shaded margins
± microcalcifications

374 pz (262 ♀; 112 ♂)
age 50.7 ± 0.7 (14-88)

469 FNAs

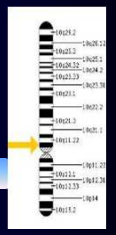
→ Biomolecular analysis

→ Pathology

Zatelli MC et al. Eur J Endocrinol. 2009 Sep;161(3):467-73

EFE 2009

THYROID CANCER PATHOGENESIS



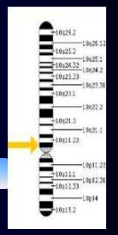
FNAB cytological analysis

- benign
- follicular lesions
  - follicular lesions of undetermined significance
  - follicular neoplasm/suspicious for follicular neoplasm
- suspicious for malignancy
- malignant
- non diagnostic

guidelines of National Cancer Institute Thyroid Fine Needle Aspiration State of the Science Conference

Baloch et al. *Diagn Cytopathol* 2008 **36** 425-437

THYROID CANCER PATHOGENESIS

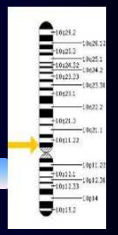


BRAF V600E mutation analysis in nodule samples according to cytological category

Cytological category	samples	BRAF+	BRAF-
benign lesions	308	6	302
follicular lesions (89)			
follicular lesions of undetermined significance	29	0	29
follicular neoplasm/suspicious	60	1	59
suspected malignant (18 PTC, 2 BC met, 2MTC)	22	10	12
malignant (45 PTC, 4 MTC)	49	31	18
inadequate	1	0	1
total	469	48	421

Zatelli MC et al. Eur J Endocrinol. 2009 Sep;161(3):467-73

THYROID CANCER PATHOGENESIS



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University of Ferrara

469
BRAFV600E
analysis

+ 48

- 41 with positive cytology → histologically confirmed as PTCs
- 7 with negative cytology → 7 hystologically confirmed as PTCs

BRAF⊕ incidence
64%

− 421

- 30 with positive cytology → **17 PTC**: 3 FA, 2 BL, 6 MTC, 2 BC
- 88 follicular lesions → **10 PTC fv**: 7 FTC, 71 FA
- 302 with negative cytology + 1 inadequate

Zatelli MC et al. Eur J Endocrinol. 2009 Sep;161(3):467-73

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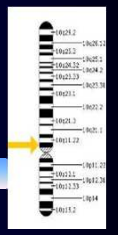
THYROID CANCER PATHOGENESIS



Statistical evaluation

	PPV	NPV	sensitivity	specificity	accuracy
Cytology	92,1%	95,9%	77,3%	98,8%	95,4%
V600E *BRAF* mutation analysis	100,0%	93,7%	64,0%	100,0%	94,4%
Both	92,9%	97,5%	86,7%	98,8%	96,9%

McNemar test
(with Yates correction)

$P < 0.01$

Zatelli MC et al. Eur J Endocrinol. 2009 Sep;161(3):467-73

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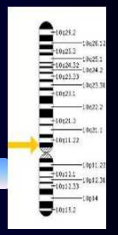
THYROID CANCER PATHOGENESIS



BRAF V600E mutation analysis

associated to FNAB cytology

significantly increases

PTC diagnostic accuracy of cytology in thyroid FNAB

of clinically and US suspicious nodules

may be added to pre-surgical risk evaluation of PTCs

Zatelli MC et al. Eur J Endocrinol. 2009 Sep;161(3):467-73

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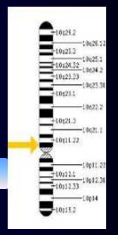
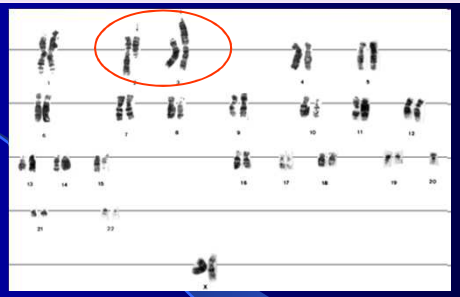
THYROID CANCER PATHOGENESIS



PAX8/PPARγ rearrangement



translocation t(2;3)(q13;p25)

fusion between PAX8 gene and PPARγ

Kroll et al. Science 2000;289:1357–1360

aberrant PPARγ protein overexpression

cell transformation

inhibition of normal PPARγ function

Gregory Powell et al. Oncogene 2004;23:3634–3641

deregulation of PAX8 function

Reddi et al. Endocrinology 2007;148:932–935

activation of known PPAR target genes

Giordano et al. Clin Cancer Res 2006;12:1983–1993

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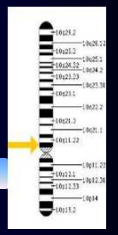
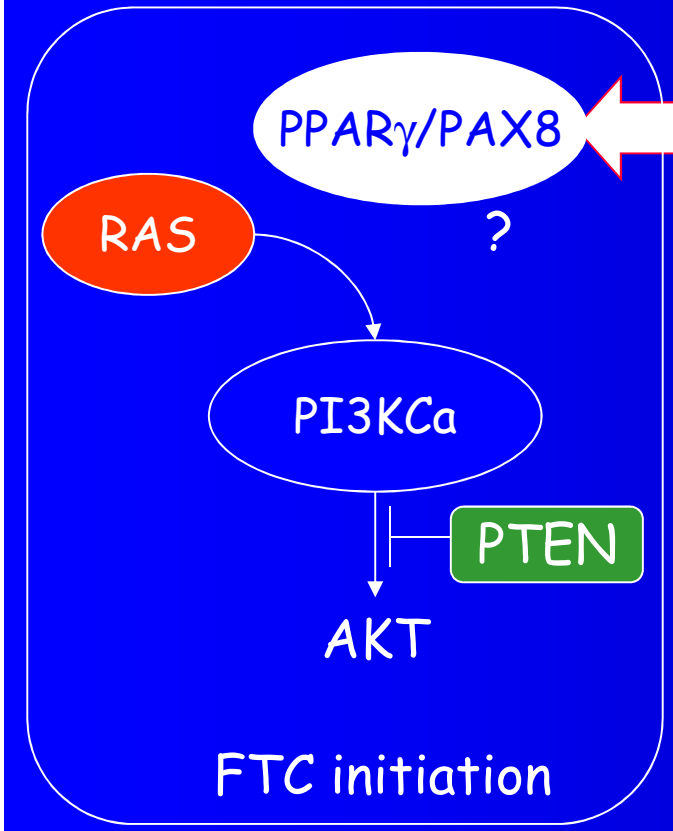
THYROID CANCER PATHOGENESIS



FOLLICULAR CARCINOMA



PPARγ/PAX8 rearrangement

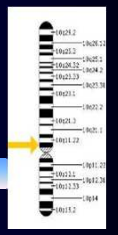
t(2;3) (q13;p25)

- ✓ 45% of FTC
- ✓ younger age
- ✓ small tumor size
- ✓ ↑ vascular invasion

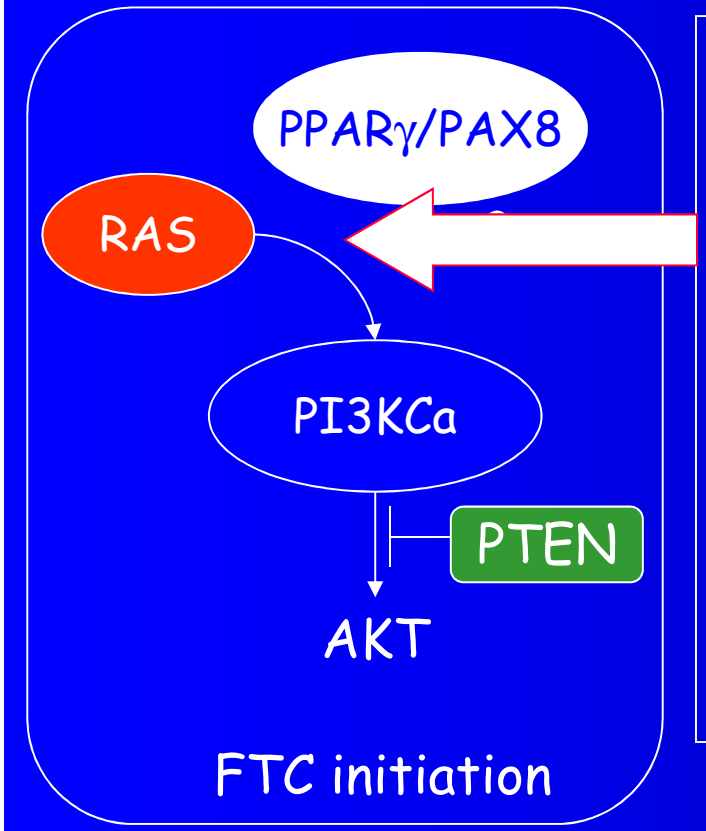
Also found in follicular adenomas and in Hurtle cell carcinomas

Nikiforova et al. Exp Rev Mol Diagn 2008, 8: 83

THYROID CANCER PATHOGENESIS



FOLLICULAR CARCINOMA



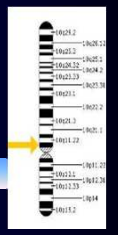
NRAS and HRAS mutations

- ✓ 30% FA and 45% of FTC
- ✓ large tumor size
- ✓ ↑distant metastases
- ✓ ↑de-differentiation and poor prognosis

Not specific !

Nikiforova et al. Exp Rev Mol Diagn 2008, 8: 83

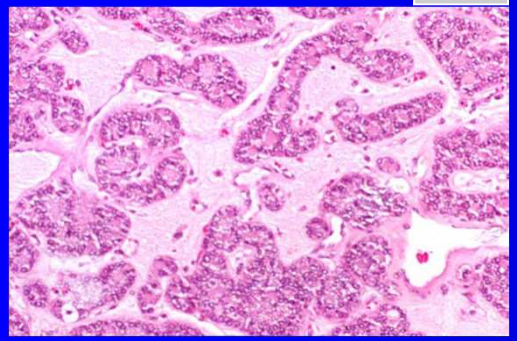
THYROID CANCER PATHOGENESIS



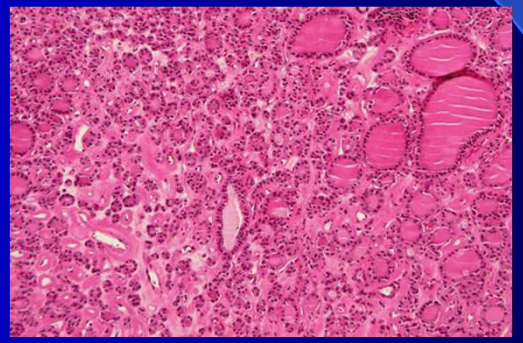
FOLLICULAR CARCINOMA

NRAS and HRAS mutations

\+



Follicular adenoma



Follicular carcinoma

prophylactic surgery?

Improved diagnostic accuracy in samples with negative or insufficient cytology

Nikiforova et al. Mod Pathol 2004, 17 (suppl. 1):77A

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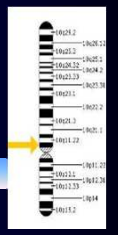
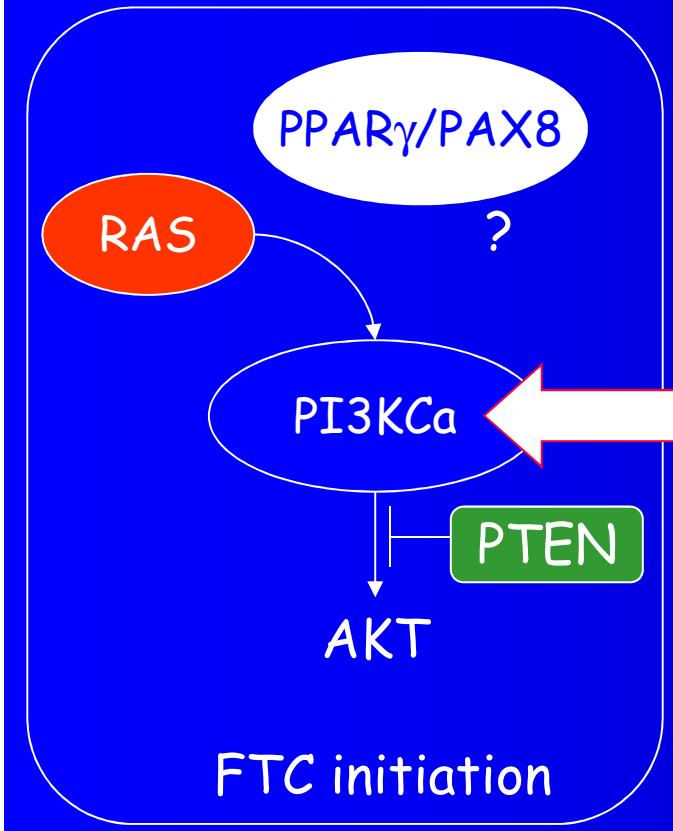
THYROID CANCER PATHOGENESIS



FOLLICULAR CARCINOMA



PI3K/Akt pathway alterations

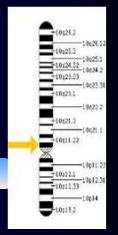
PIK3CA mutations: 23% ATC and 8% FTC

PTEN LOH: 7% FA and 27% of FTC

Lack of large studies

Nikiforova et al. Exp Rev Mol Diagn 2008, 8: 83

THYROID CANCER PATHOGENESIS



ANAPLASTIC CARCINOMA

p53 inactivating point mutations

- ✓highly prevalent in ATC and PDC

Ito et al. 1992 Cancer Res 52:1369–1371
Fagin et al. 1993 J Clin Invest 91:179–184

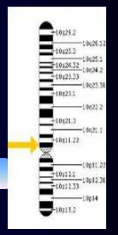
- ✓are not directly tumorigenic

  ⤷ cooperation with other oncogenes is necessary for malignancy

- ✓loss of differentiated phenotype

Battista et al. 1995 Oncogene 11:2029–2037

THYROID CANCER PATHOGENESIS



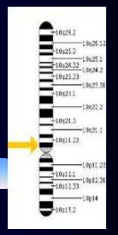
ANAPLASTIC CARCINOMA

Mutations in anaplastic thyroid carcinomas

References	Ras	BRAF	TP53	Catenin[a], beta 1	PIK3CA	Axin	APC	PTEN
Fagin *et al.* (1993)			5/6					
Donghi *et al.* (1993)			5/7					
Zou *et al.* (1993)			1/5					
Zedenius *et al.* (1996)			1/4					
Garcia-Rostan *et al.* (1999)				19/31				
Garcia-Rostan *et al.* (2003)	15/29							
Fukushima *et al.* (2003)	2/7	0/7						
Namba *et al.* (2003)		2/6						
Nikiforova *et al.* (2003)		3/29						
Soares *et al.* (2004)		6/17						
Begum *et al.* (2004)		8/16						
Kurihara *et al.* (2004)				1/22		18/22	2/22	
Quiros *et al.* (2005)	1/8	5/8						
Garcia-Rostan *et al.* (2005)					16/70			
Takano *et al.* (2007a)		4/20						
Mitsiades *et al.* (2007)		1/7						
Hou *et al.* (2007) and Liu *et al.* (2008)	4/50	14/49			6/50			8/48
Santarpia *et al.* (2008)	2/36	9/36			5/36			2/36
Costa *et al.* (2008)	13/36	9/36						
Total (%)	37/166 (22%)	61/231 (26%)	12/22 (55%)	20/53 (38%)	27/156 (17%)	18/22 (82%)	2/22 (9%)	10/84 (12%)

Smallridge et al Endocrine-Related Cancer 2009,16:17–44

THYROID CANCER PATHOGENESIS



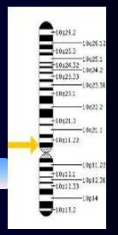
ANAPLASTIC CARCINOMA

Cytogenetic analyses in patients with anaplastic thyroid cancer

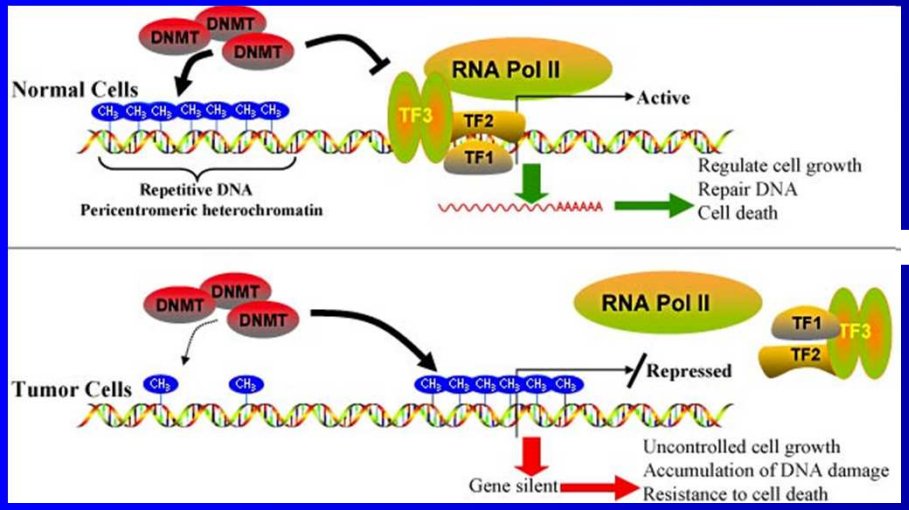
References	Patients (*n*)	Cell lines (*n*)	Gains	Losses	Both	Chromosomes
Jenkins *et al.* (1990)	2	–				Complex clonal karyotype; loss of eight (both); x; y −2, −4, −8, −9, +11, −13, +14, −14, +15, −17, −19, −20, −21, −22, −x, −y
Hemmer *et al.* (1999)	13	–	10/13	3/13	2/13	+7p (31%); +8q (23%); +9q (23%)
Wilkens *et al.* (2000)	9	2				Gains in 14 chromosomes; losses in eight chromosomes; most common: 5p amplified; 8p, 8q gains or losses
Kitamura *et al.* (2000)	21					Allelic losses 16/21; Losses: 1q (40%); 9p (58%) ; 11p (33%); 11q (33%); 17p (44%); 17q (43%); 19p (36%); 22q (38%)
Wreesmann *et al.* (2002)	15	–				*Early* Gains: 5, 8, 19; losses: 8, 22; *intermediate:* gains:1, 6, 9, 17, 20; losses: 1, 2, 6, 13 *late*: gains: 3p, 11q; losses: 5q
Miura *et al.* (2003)	10	1	5/10	2/10		Gains: 1, 4, 5, 6, 7, 10, 14, 16, 19, 20, 21, x; losses: 3p; 9q
Rodrigues *et al.* (2004)	7	–				Gains in all chromosomes; losses less common; high amplification in: 3p, 5p, 6p, 9p, 12p, 14q, 18p
Rodrigues *et al.* (2007)	–	3				Amplifications: 3q24; 5p; 7p; 7q; 12p; 14q; 20
Lee *et al.* (2007)	–	8				Frequent: gains: 8q; 11q; 19; 20q; losses: 4q; amplification: 8q
Lee *et al.* (2008)	27					Gains: 1q21; 6p22-p21; 7q11.22-11.23; 11q13; 12q13; 16p11.2; 17q21; 19p13; 19q13. 1-q13.2; 20q11.2; 20q13.12; 22q11.21; 22q.13.1 Losses: 4q12-q13.1; 4q28.3; 13q21.2-q21.31

Smallridge et al Endocrine-Related Cancer 2009,16:17–44

THYROID CANCER PATHOGENESIS



Aberrant gene methylation



→ aberrant gene transcription

→ tumor suppressor genes → tumorigenesis

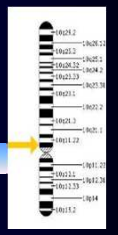
→ thyroid-specific genes

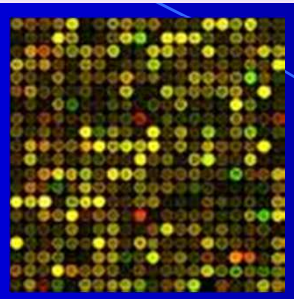
→ radioiodine treatment failure

→ clinical progression

EFE 2009

THYROID CANCER PATHOGENESIS





Microarray analysis

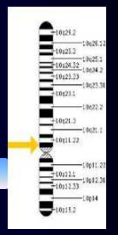
gene expression radiation signatures

DNA repair pathway

susceptibility to develop
radiation-induced cancer

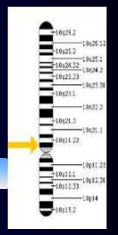
Detours et al. Curr Opin Endocrinol Diabetes Obes 15:440–445

THYROID CANCER PATHOGENESIS



Why understanding
thyroid cancer pathogenesis
is so important?

THYROID CANCER PATHOGENESIS



Receptor tyrosine kinase inhibitors in clinical trials in thyroid cancer

Name	Other names	Tyrosine kinase targets (only those $IC_{50} < 1\ \mu M$)	Clinical development
Imatinib mesylate (Novartis)	Gleevec, glivec, STI571	ABL, KIT, PDGFR	Approved for CML, GIST
Gefitinib (AstraZeneca)	ZD1839, iressa	EGFR	Approved in some countries for NSCLC
Axitinib (Pfizer)	AG-013736	VEGFR-1, -2, -3, PDGFR, KIT	Investigational
Vandetanib (AstraZeneca)	Zactima, ZD6474	EGFR, RET, VEGFR-2, -3	Investigational
Sunitinib (Pfizer)	Sutent, SU11248	VEGFR-2, PDGFR, KIT, FLT3, RET, FGFR-1, CSF-1R	Approved for metastatic renal carcinoma and imatinib-resistant GIST
Sorafenib (Bayer)	Nexavar, BAY 43-9006	RAF, BRAF, P38, VEGFR-1, -2, -3, PDGFR, FLT3, RET, KIT, FGFR-1	Approved for metastatic renal carcinoma
Motesanib diphosphate (Amgen)	AMG 706	VEGFR-1, -2, -3, KIT, RET, PDGFR, FLT3	Investigational
XL184 (Exelixis)	—	RET, VEGFR-2, MET	Investigational
Pazopanib (GlaxoSmithKline)	GW-786034	VEGFR-1, -2, -3, PDGFR, KIT, FGFR-1	Investigational

ABL, Abelson murine leukaemia viral (v-abl) oncogene homologue; PDGFR, platelet-derived growth factor receptor; KIT, stem-cell factor receptor; EGFR, epidermal growth factor receptor; VEGFR, vascular endothelial growth factor receptor; FLT3, fms-related tyrosine kinase; RET, rearranged during transfection; FGFR, fibroblast growth factor receptor; MET, hepatocyte growth factor receptor; CSF-1R, colony-stimulating factor receptor.

**CML = chronic myelogenous leukaemia, GIST = gastro intestinal stromal tumours; NSCLC = non small cell lung carcinoma.

Castellone et al, Best Pract Res Clin Endocrinol Metab 2008, 22: 1023–1038

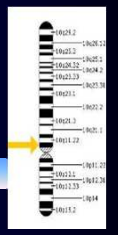
THYROID CANCER PATHOGENESIS

In conclusion

- much has been discovered concerning thyroid cancer pathogenesis
- preclinical studies help in characterizing the activity of new drugs and to validate their targets
- patients enrolled in clinical studies need to be genotyped for the compound target

New opportunities to design clinical trials based upon tumor molecular profiling and preclinical studies of potentially synergistic combinatorial novel therapies

Endocrine Molecular Lab



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THANK YOU!

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