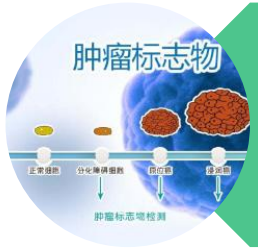
二代测序技术在甲状腺肿瘤中检测探索

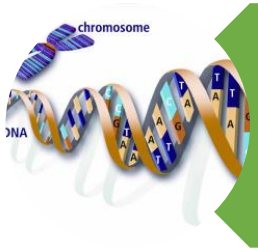
中山大学肿瘤防治中心
王海云

内容

基因组学与二代测序技术背景介绍



肿瘤分子标志物

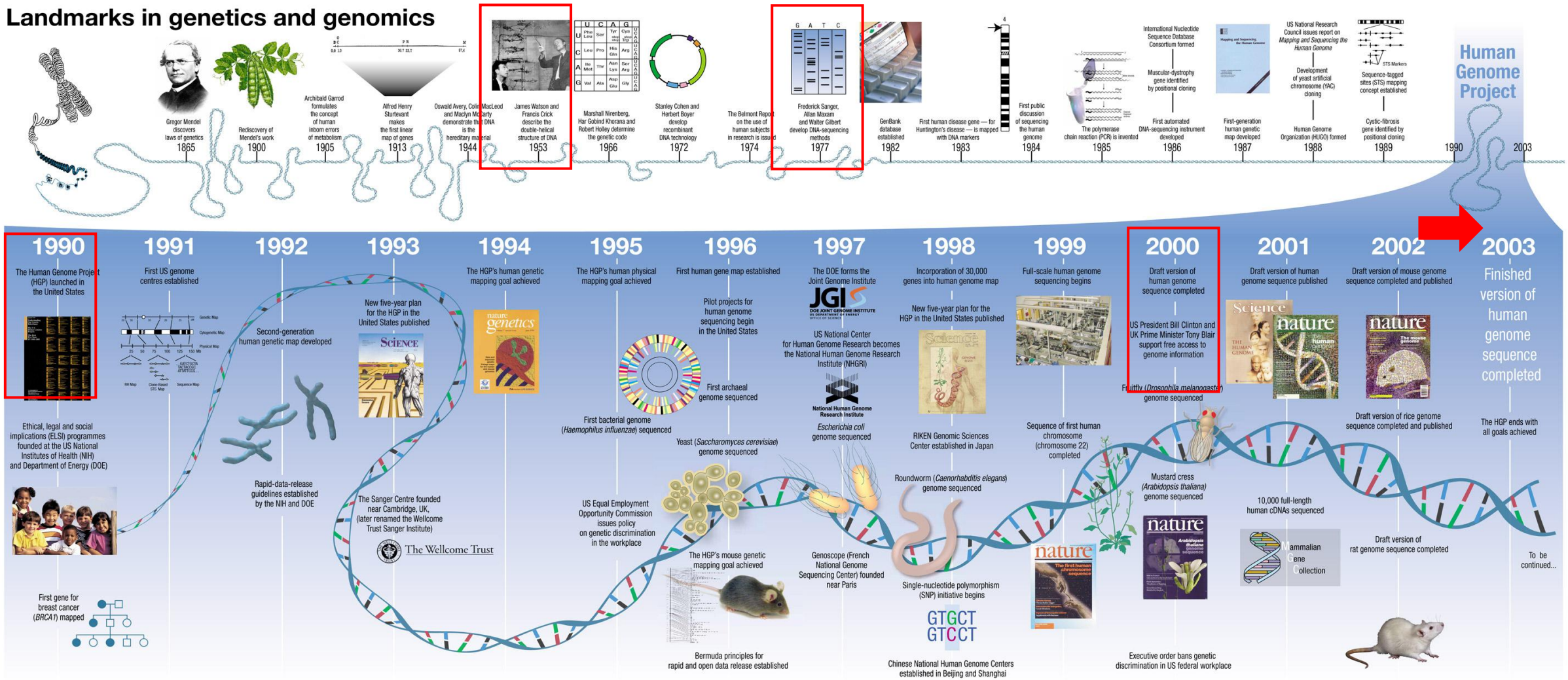


甲状腺肿瘤分子检测探索

内容

一 基因组学与二代测序技术背景介绍

遗传学和基因组学发展历程



DESIGN BY DARRYL LEJA
PEAS COURTESY J. BLAMIRE, CITY UNIV. NEW YORK; WATSON & CRICK COURTESY A. BARRINGTON BROWN/SPL; *SCIENCE* COVERS COURTESY AAAS

Hikmet Geçkil

国际肿瘤基因组计划的研究目标

- 全面解析与鉴别重要癌肿的遗传学基础
- 全面鉴定重要癌肿的基因组学变异，转录组学及表观遗传学基因图谱等

TCGA肿瘤数据分享平台

NIH NATIONAL CANCER INSTITUTE

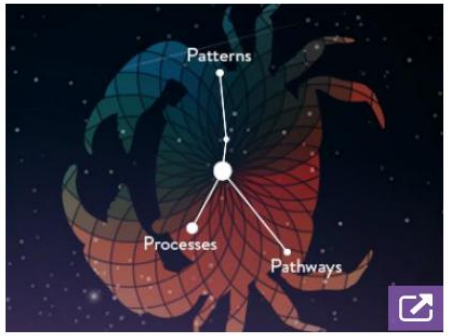
The Cancer Genome Atlas Program

Common Cancer Types

- Bladder Cancer
- Breast Cancer
- Colon and Rectal Cancer
- Endometrial Cancer
- Kidney Cancer
- Leukemia
- Liver Cancer
- Lung Cancer
- Melanoma
- Non-Hodgkin Lymphoma
- Pancreatic Cancer
- Prostate Cancer
- Thyroid Cancer

多种数据类型

基因组学数据
蛋白质数据
表观遗传学数据
转录组学数据
临床诊断



涵盖33个癌肿，共2万多例肿瘤和正常样本

头颈肿瘤

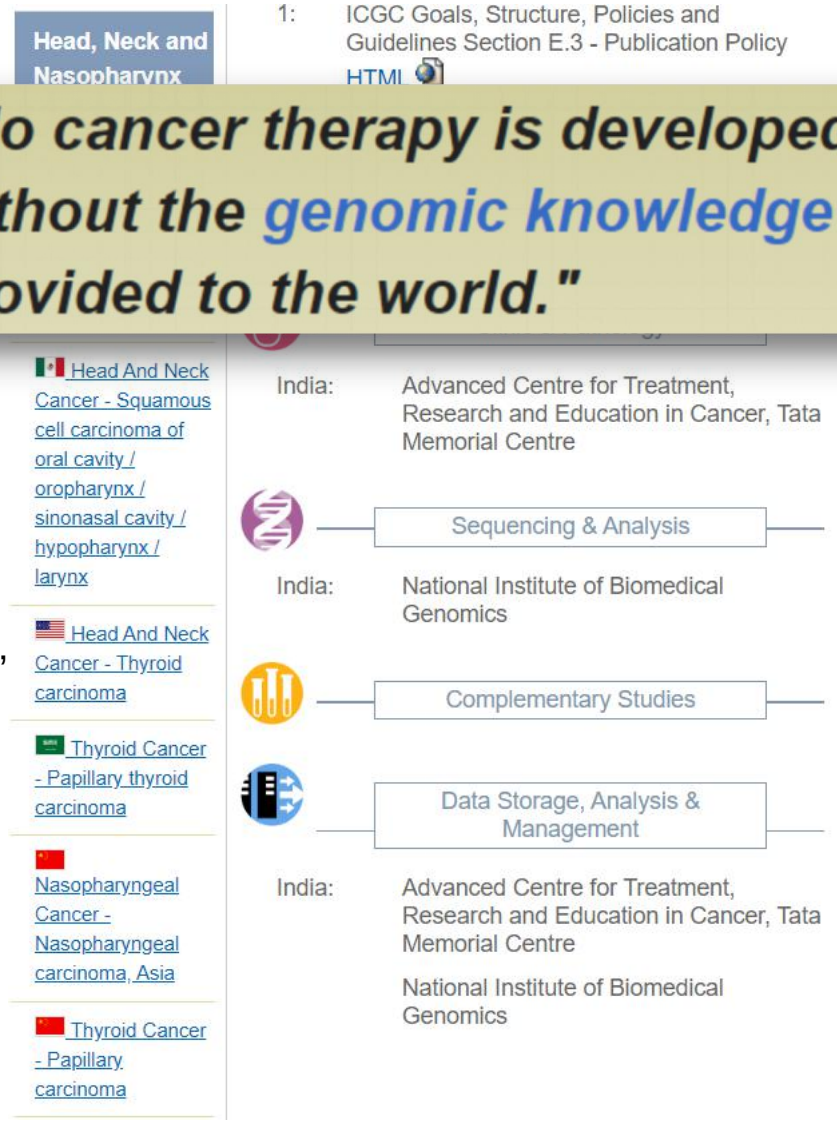
Oral cancer
Lip cancer
Oropharyngeal cancer
NPC
Thyroid cancer

ICGC肿瘤数据分享平台

中山大學 肿瘤防治中心 SUN YAT-SEN UNIVERSITY CANCER CENTER SYSUCC SINCE 1964

International Cancer Genome Consortium

伦理委员会
组织样本库和临床结果解读小组
技术组包括变异验证小组，表观遗传组，录组，以及文库小组等
生物信息分析小组



头颈肿瘤主要有：

Oral cancer
Head and neck c squamous cell carcinoma
Oral Cavity cancer
Oropharyngeal cancer
NPC
Thyroid cancer

二代测序技术

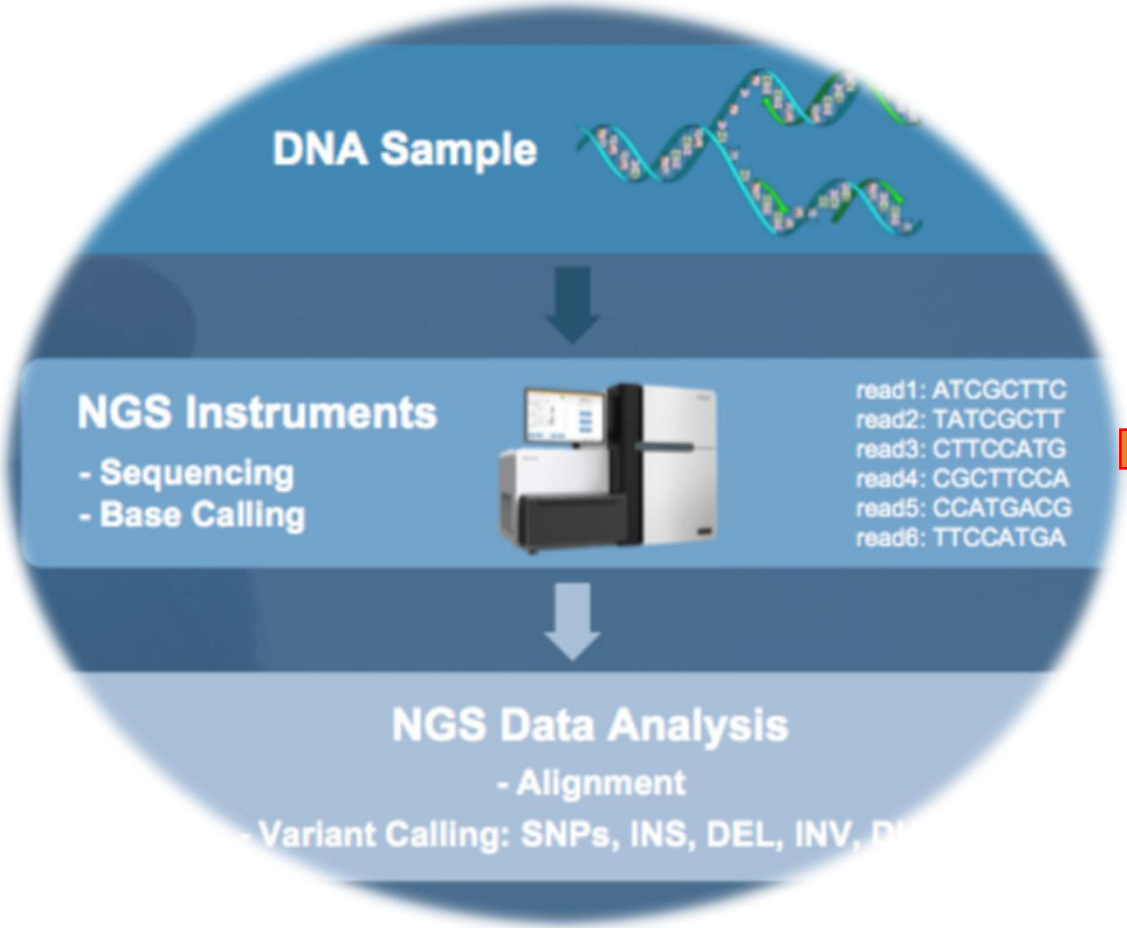
DNA变异检测：

全基因组测序(the whole genome sequencing)

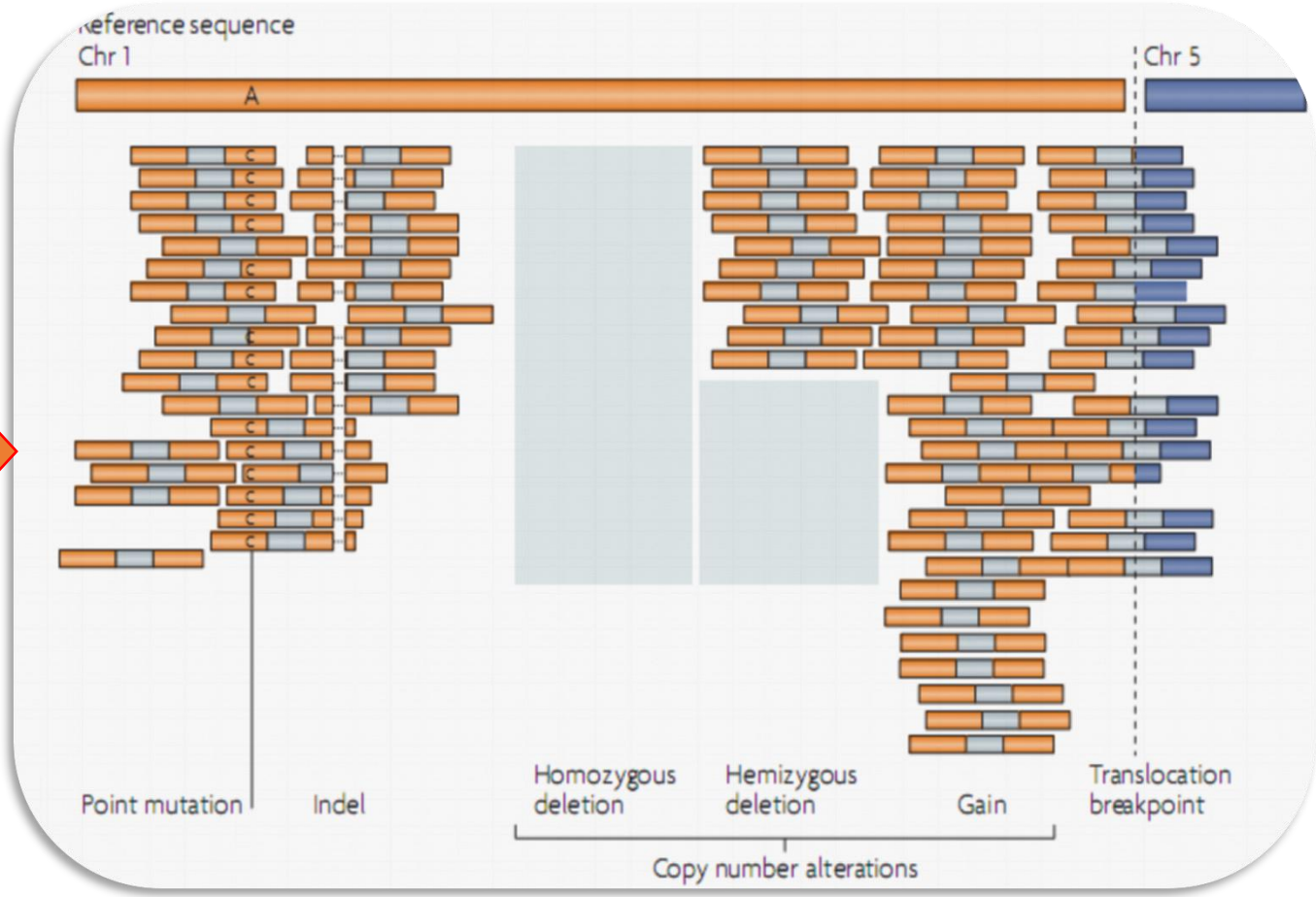
全外显子测序(the exome sequencing)

目标靶区测序(the targeted region sequencing)

体细胞DNA变异检测



(Matthew Meyerson et.al, nature reviews genetics,2010)



- Point mutations (nucleotide substitutions)
- Small coding insertions/deletions (indels)
- Copy number alterations (CNAs)
- Chromosomal rearrangements

二代测序技术

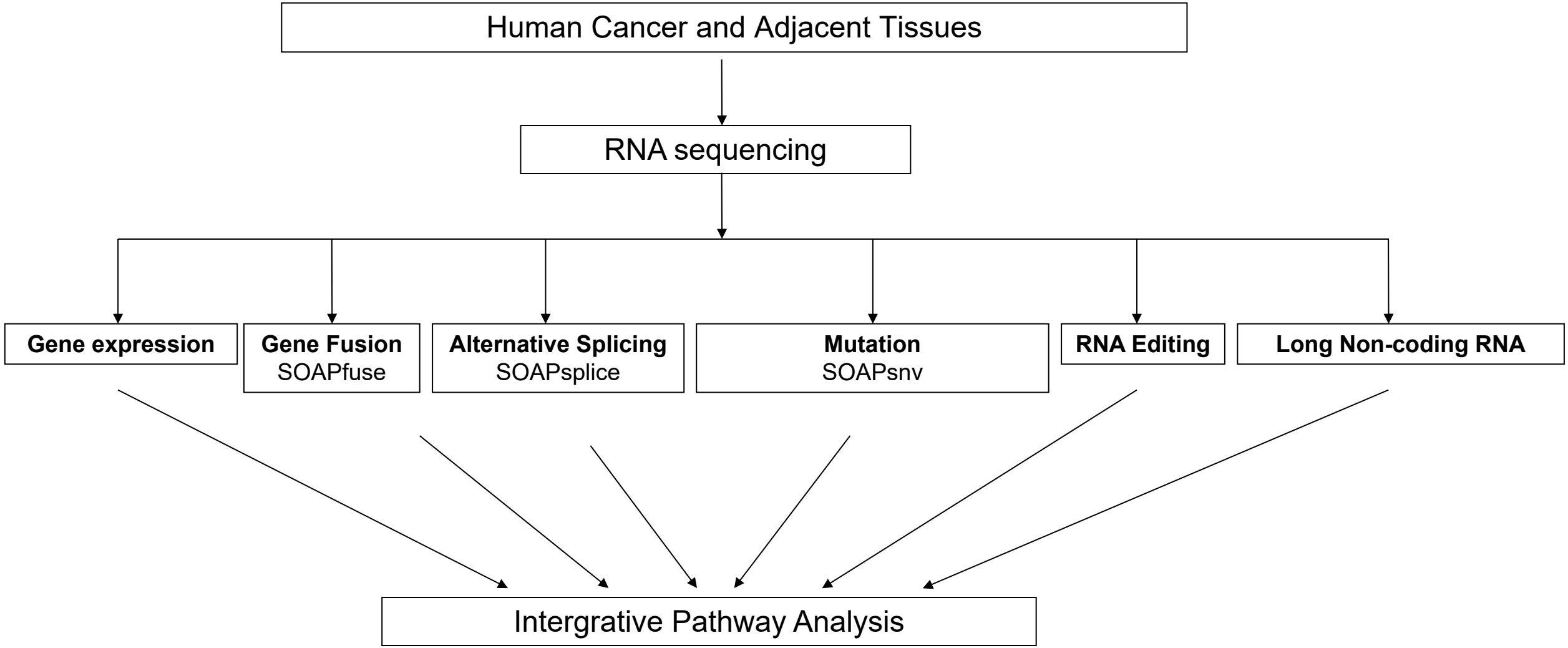
RNA变异检测：

转录组测序(Transcriptome Sequencing)

表达谱测序(Expression sequencing)

小RNA和长非编码RNA测序(miRNA and lncRNA sequencing)

肿瘤RNA变异检测



Systematic Cancer Transcriptome Analysis

二代测序技术

表观调控分析检测：

DNA甲基化

全基因组重亚硫酸盐测序(WGBS)
甲基化 DNA 免疫共沉淀测序(MeDIP-seq)
基于双酶切消化的重亚硫酸盐测序(dRRBS)

DNA羟甲基化

HMST-Seq
羟甲基化 DNA 免疫共沉淀测序

RNA甲基化

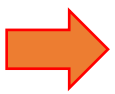
组蛋白修饰

染色质免疫共沉淀测序(ChIP-Seq)

常见癌肿的基因变异情况

- 体细胞突变
- 染色体重排
- 小片段插入/缺失
- 遗传性突变

Cancer type	Somatic mutations	Structural rearrangements	Insertions/Deletions	Hereditary mutations
Melanoma	HRAS, BRAF, RAS, CTNNB1, GNA11, GNAQ, KIT, MEK1, NRAS, MMAC1, PTEN, GRIN2A, PREX2, RAC1, PPP6C, STK19, SNX31, TACC1, ARID2, CDK4, MITF	CDKN2, HMGI-C, RAF	MMAC1, PTEN, BRAF	MAP2K1, MAP2K2, CDK4
Lung Cancer	TP53, KRAS, STK11, EGFR, ALK, BRAF, RAS, AKT1, DDR2, HER2, MEK1, NRAS, PIK3CA, PTEN	RET, ROS1, ALK, EML4, NTRK1	EGFR, FHIT, FRA3B, FGFR1, HER2	EGFR, BRAF, RAS, KRAS, TP53
Liver Cancer	CTNNB1, GNMT, p57, IN1	GNMT	E1B, GNMT, p57	ARID1A, ARID1B, ARID2, MLL, MLL3, TP53, CTNNB1, EGFR, GNMT, IN1
Breast Cancer	FBXW7, AKT2, PI3KCA, CCND1, ERBB2, NTRK1, SMAD2, MAP2K4, AKT1, ESR1, FGFR1, FGFR2, HER2, PIK3CA, PTEN	BRCA1, BRCA2, TP53, PIK3CA, CHEK2	BRCA1	TP53, BRCA1, BRCA2, PTEN, AKT1, TP43, TBX3, RUNX1, CBFB
Colorectal Cancer	CTNNB1, BAX, FBXW7, PI3KCA, FES, KRAS, NRAS, SMAD2, SMAD4, TGFBR1, TGFBR2, MAP2K4, PTNP1, AKT1, PTEN, BRAF, PIK3CA	ALK	APC-L1	FAP, AXIN2, MSH2, MLH1, MSH6, PMS2, HNPCC, MUTYH, PIK3CA, IRS2, IRS4, PTEN, RHEB
Ovarian	FBXW7, AKT2, ERBB2, TGFBR1, TGFBR2, BRAF, KRAS, PIK3CA, PTEN	ARID1A, BRCA1	MMP-1, BRCA1, BRCA2, MLPA, MAPH	STK11, BRCA1, BRCA2
Pancreatic	KRAS2, N-RAS, MAP2K4, CDKN2A, TP53, SMAD4, BRCA2	TP53, SMAD4, INK4A	CDKN2A, TP53	STK11, CDKN2A
Thyroid	NTRK1, BRAF, KRAS, RET	RET/PTC, ELE1, AKAP9, PTEN,	PTEN	RET, NTRK1, BRAF

甲状腺癌 

肿瘤基因组学进入大数据时代

Cell

Article

An Integrated TCG
Clinical Data Reso

Article

Cell

Cell. 2018 Apr 5;173(2):321-337.e10. doi: 10.1016/j.cell.2018.03.035.

Oncogenic Signaling Pathways in The Cancer Genome Atlas.

Sanchez-Vega F, Mina M, Armenia J, Chatila WK, Luna A, La KC, Dimitriadoy S, Liu DL, Kantheti HS, Saghafinia S, Chakravarty D, Daian F, Gao Q, Bailey MH, Liang WW, Foltz SM, Shmulevich I, Ding L, Heins Z, Ochoa A, Gross B, Gao J, Zhang H, Kundra R, Kandoth C, Bahceci I, Dervishi L, Dogrusoz U, Zhou W, Shen H, Laird PW, Way GP, Greene CS, Liang H, Xiao Y, Wang C, Iavarone A, Berger AH, Bivona TG, Lazar AJ, Hammer GD, Giordano T, Kwong LN, McArthur G, Huang C, Tward AD, Frederick MJ, McCormick F, Meyerson M; Cancer Genome Atlas Research Network, Van Allen EM, Cherniack AD, Ciriello G, Sander C, Schultz N.

⊕ Collaborators (725)

多学科，多个研究中心合作成为肿瘤大数据时代的必备条件

内容

二 肿瘤分子标志物

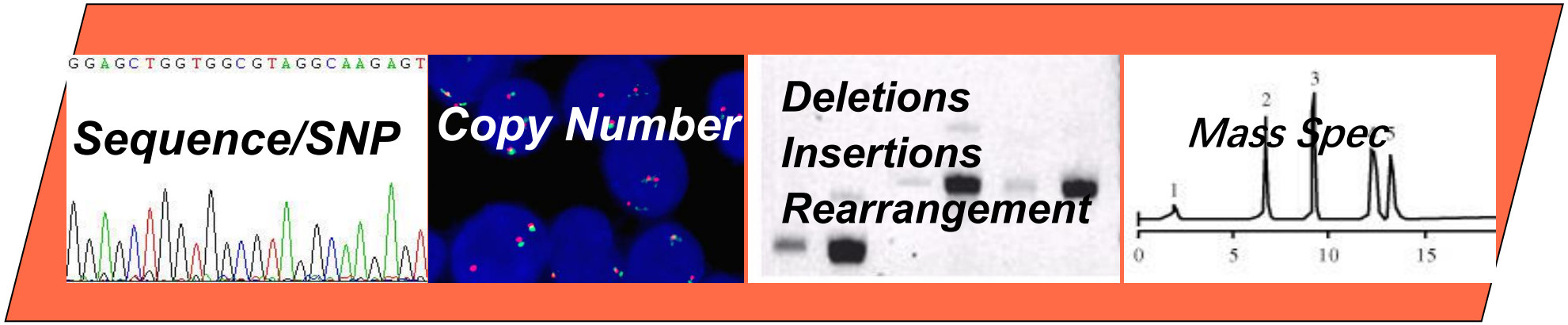
肿瘤标志物
（tumor markers, TM）

- 1978年NCI提出肿瘤标志物
- 1979年确认并开始使用
- TM是表示肿瘤存在并反映其一定的生物特性的生化物质。
- TM主要是指那些在血液、体液及组织中可检测到的与肿瘤相关的物质，达到一定的水平时能揭示某些肿瘤的存在。
  - 甲胎蛋白（AFP）
  - 癌胚抗原（CEA）
  - 糖类抗原（CA50、CA125、CA15-3、CA199、CA72-4）
  - ß2-微球蛋白（ ß2-MG）
  - 前列腺特异抗原（PSA）

肿瘤分子标志物的临床应用

- ✓ 筛选 （Screen）
- ✓ 诊断 （Diagnosis）
- ✓ 预后分析（Prognosis)
- ✓ 复发监测（Detection of relapse）
- ✓ 治疗指导（Monitor of therapy）

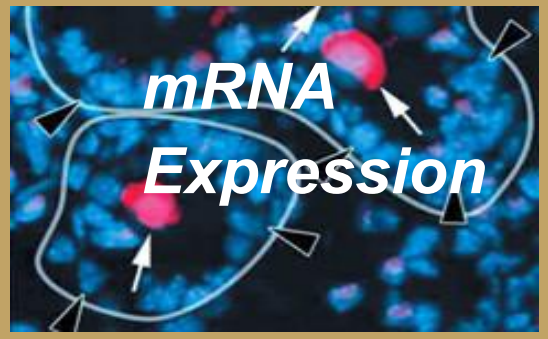
DNA水平（genomics）

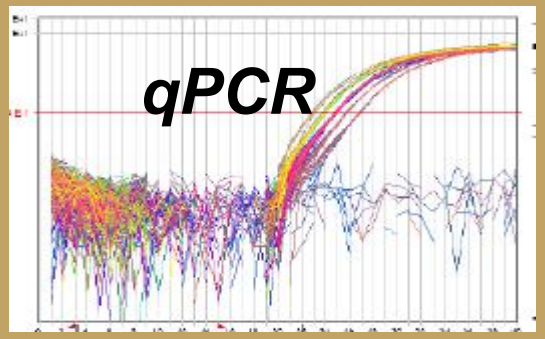


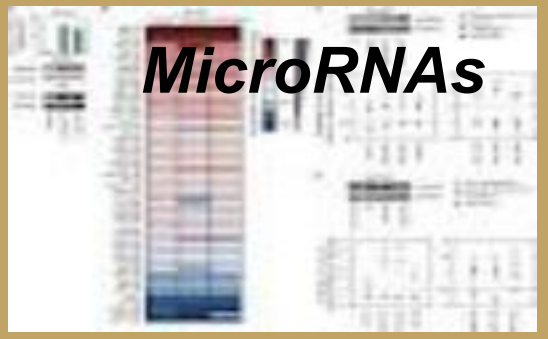
- **DNA检测技术**
  - --测序　　--FISH　　--高通量质谱
  - --PCR　　--芯片技术

- **DNA改变的类型**
  - --SNP
  - --大片段或小片段的插入或缺失
  - --基因重排
  - --拷贝数的改变

RNA水平（Transcriptomics）







- **检测RNA技术**

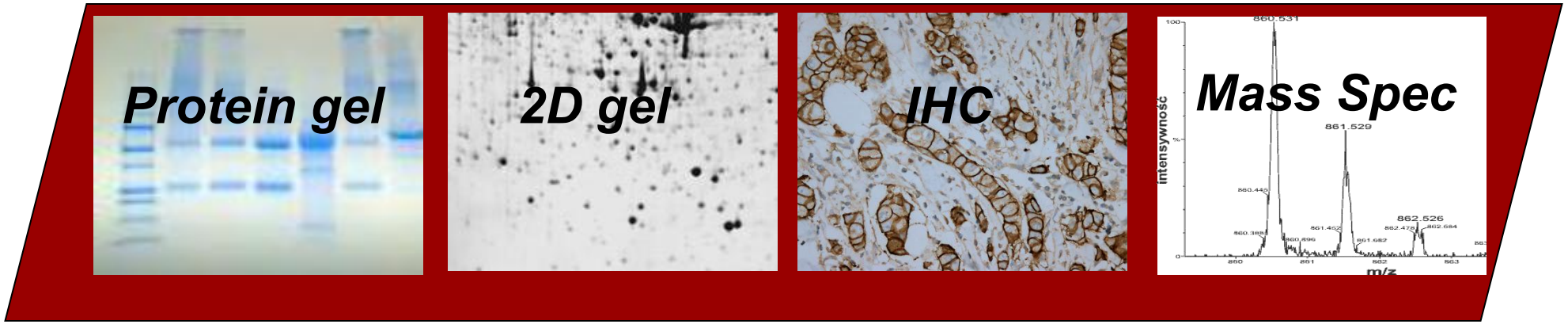
  --微阵列　　--（荧光）原位杂交

  --RT-PCR　　--Northern Blot

- **RNA表达改变形式**

  --增加　　--减少　　--缺失

Protein水平（Proteomics）



- **检测蛋白技术**

  --凝胶分析

  --IHC

  --质谱技术

- **检测范围**

  --蛋白表达、含量、分子量

可用于分子标记物检测的标本种类

- 组织学
  - 石蜡组织
    - 易获得的标本
    - 难获得的DNA
  - 新鲜组织
    - 易获得的标本
    - 高质量的DNA
- 细胞学
  - 细针穿刺收集
  - 脱落细胞
- 液体活检标本

内容

三　甲状腺肿瘤分子检测探索

甲状腺癌---发病率增高最快的实体癌

2010年韩国癌症统计报告：

甲状腺癌上升至癌症首位

美国1989 年～2009年

甲状腺癌发病率增长4.99倍

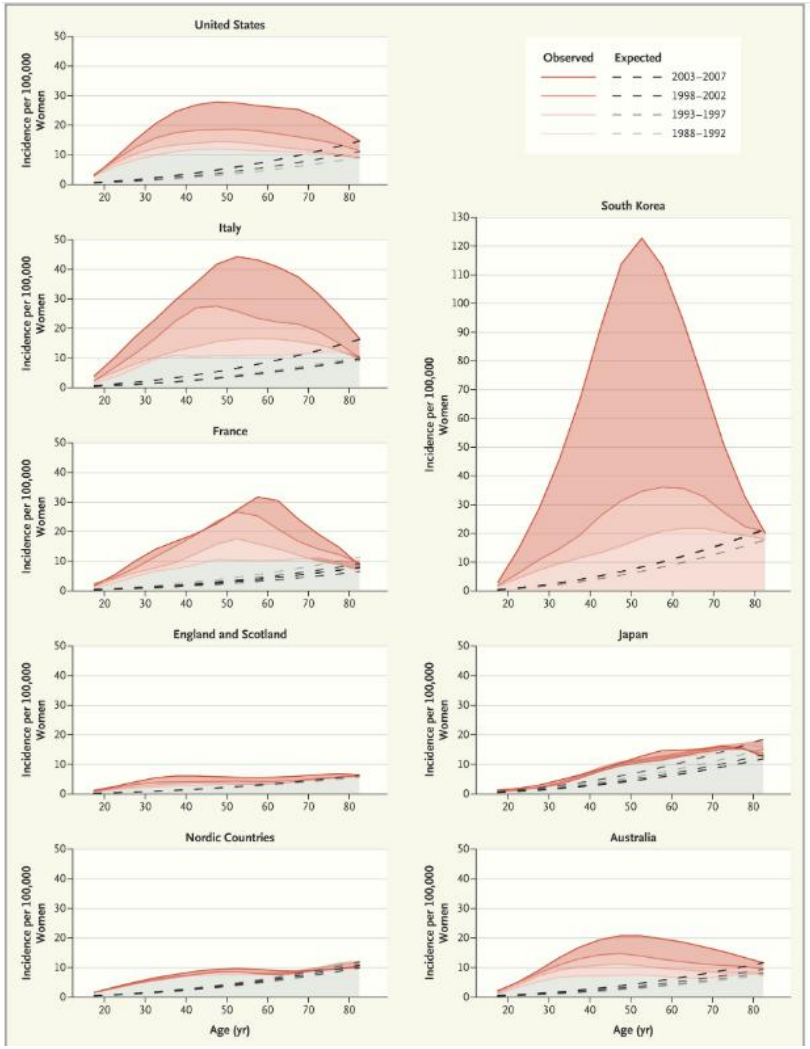
2012年中国卫生部统计报告

甲状腺癌上升至女性恶性肿瘤第3位

2011年北京：甲状腺癌患病率9年间增长了225.2%

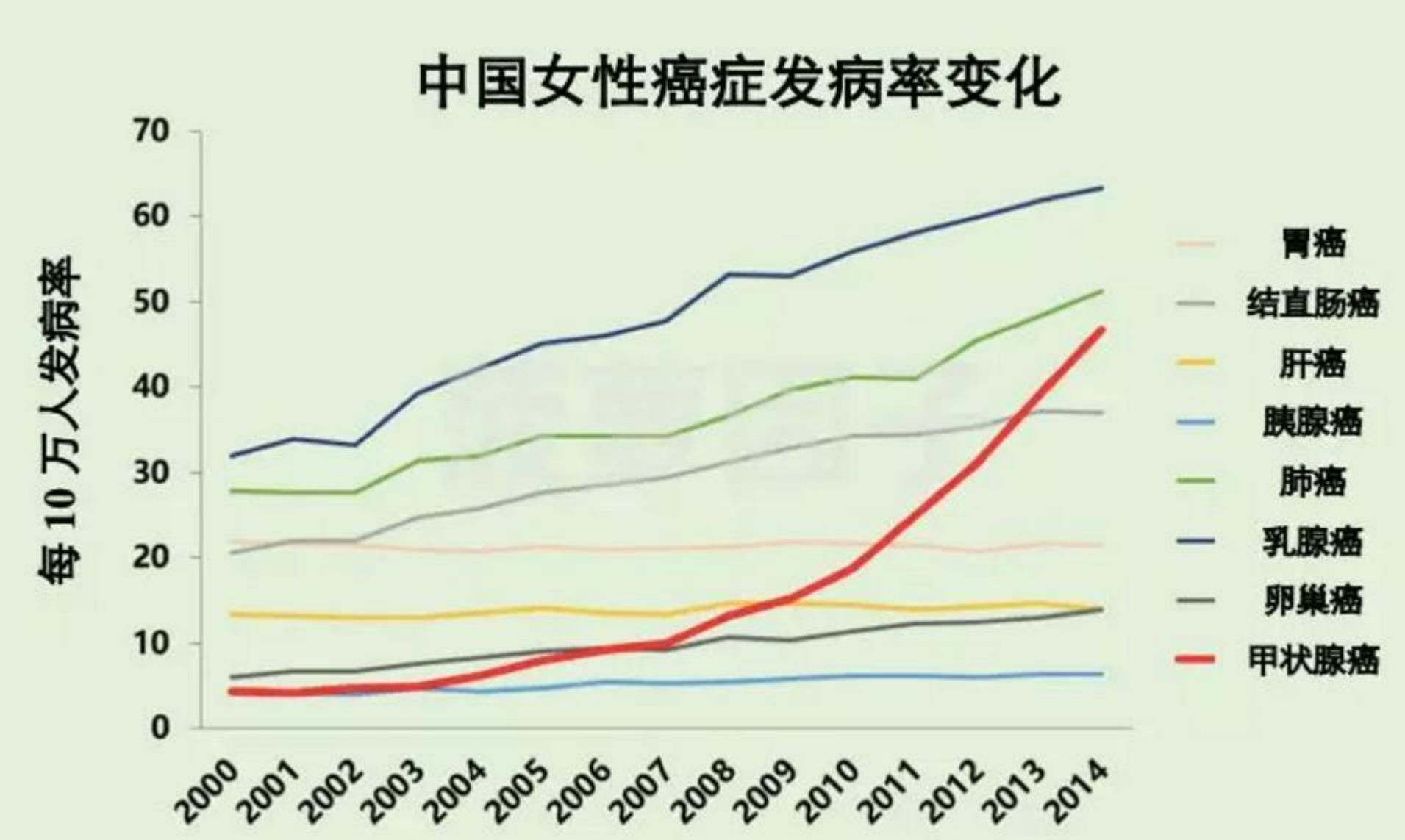
2010年上海：甲状腺癌上升至女性恶性肿瘤第5位

1. Cooper DS, et al. Thyroid, 2009, 19(11): 1167-1214.
2. 2011年度北京市卫生与人群健康状况报告
3. 2010年度上海市恶性肿瘤报告
4. 2010年韩国国民癌症统计报告



N Engl J Med. 2016 Aug

中国女性甲状腺癌发病率上升最快



甲状腺癌类型	百分比（%）	高发年龄（岁）	生存率
乳头状癌	85～90	20-40	极高
滤泡状癌	5～10	40-50	高（很多需要系统性治疗，包括放射性碘-131）
未分化癌	0～5	＞ 50	低
髓样癌	0～5	通常为老年人，但遗传性肿瘤会在年轻人中发生	个体差异大，遗传性髓样癌预后更差

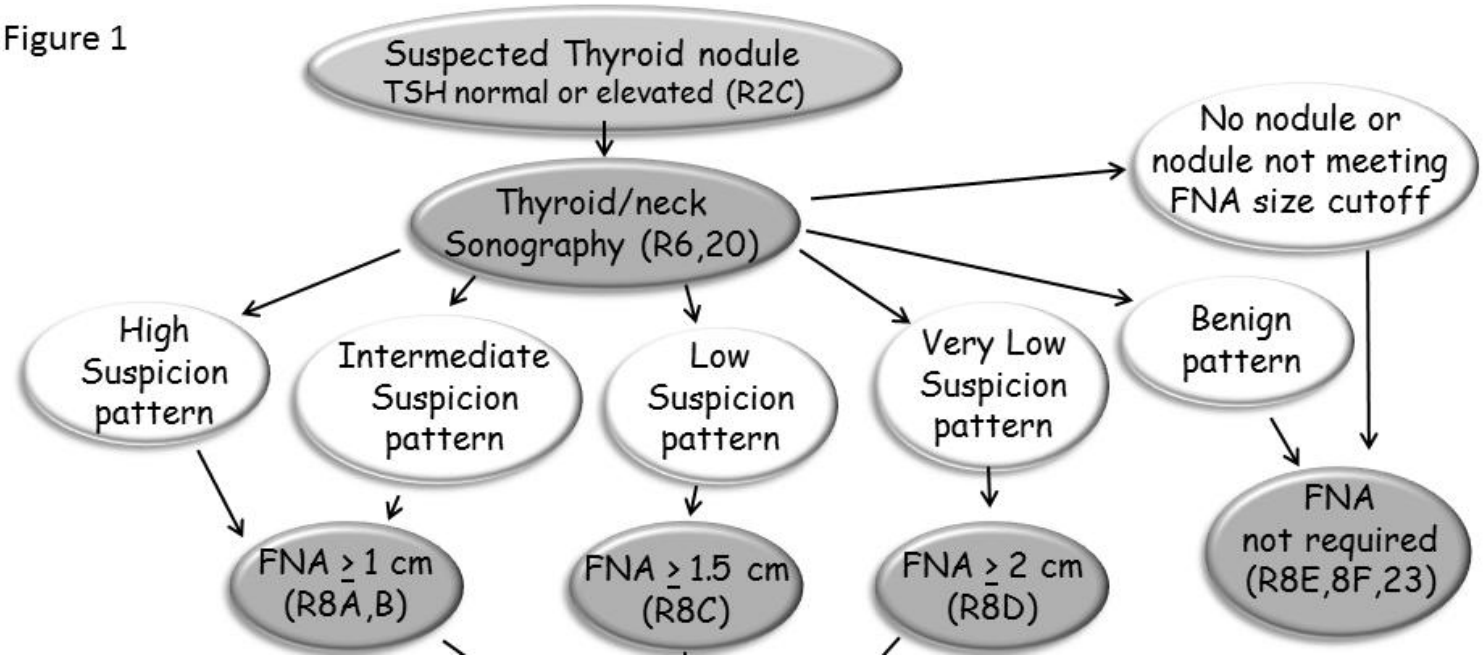
PTC发病率持续上升

	Papillary thyroid		Prostate		Lung		Colorectal			Ovarian	
Year	Overall	Males	Females	Males	Overall	Males	Females	Overall	Males	Females	Females
2009	12.1	5.8	18.2	146.7	54.7	64.9	47.5	42.4	48.3	37.7	12.5
APC	7.00%	6.20%	7.30%	−2.1%	−1.1%	−2.0%	−1.2%	−2.4%	−2.7%	−2.1%	−1.4%
2010	12.9	6.2	19.6	143.7	54.1	63.6	46.9	41.4	47	36.9	12.3
2011	13.8	6.5	21	140.7	53.5	62.3	46.4	40.4	45.8	36.1	12.1
2012	14.8	7	22.6	137.8	52.9	61.1	45.8	39.5	44.5	35.3	11.9
2013	15.8	7.4	24.2	135	52.3	59.9	45.3	38.5	43.3	34.6	11.8
2014	16.9	7.8	26	132.2	51.8	58.7	44.7	37.6	42.2	33.8	11.6
2015	18.1	8.3	27.9	129.5	51.2	57.6	44.2	36.7	41	33.1	11.4
2016	19.4	8.9	29.9	126.8	50.6	56.4	43.6	35.9	39.9	32.4	11.3
2017	20.8	9.4	32.1	124.2	50.1	55.3	43.1	35	38.8	31.7	11.1
2018	22.2	10	34.5	121.6	49.5	54.2	42.6	34.2	37.8	31	10.9
2019	23.8	10.6	37	119.1	49	53.2	42.1	33.4	36.8	30.4	10.8

2015年美国甲状腺协会（ATA）成人甲状腺结节与分化型甲状腺癌指南

2015 American Thyroid Association Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer

Figure 1

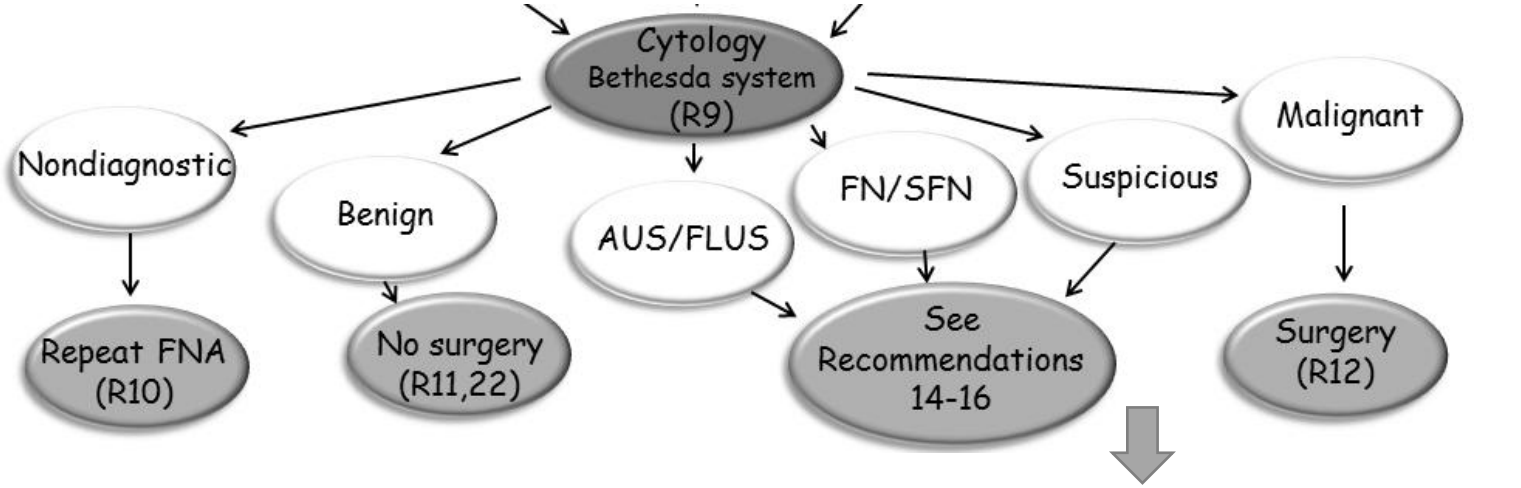


FNA

- 最早报道1843年
- 1967年，北欧学者详尽描述了各种甲状腺炎的细胞学表现
- 1980s，3位美国细胞病理学家进行5000例FNA检查，提出细胞病理学诊断标准
- 术前评估甲状腺结节良恶性时，FNAB是灵敏度和特异性最高的方法

2015年美国甲状腺协会（ATA）成人甲状腺结节与分化型甲状腺癌指南

2015 American Thyroid Association Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer



34% (range 14–48%) 手术后为恶性

2007年NCI在Bethesda制度了FNA的形态学标准：Bethesda classification scheme

甲状腺细胞病理学Bethesda 报告系统（TBSRTC）

中山大學 肿瘤防治中心 SUN YAT-SEN UNIVERSITY CANCER CENTER

The Bethesda System for Reporting Thyroid Cytopathology: Recommended Diagnostic Categories*

I. **Nondiagnostic or Unsatisfactory** 标本无法诊断或不满意：1～4%
- Cyst fluid only
- Virtually acellular specimen
- Other (obscuring blood, clotting artifact, etc)

II. **Benign** 良性： 0～3%
- Consistent with a benign follicular nodule (includes adenomatoid nodule, colloid nodule, etc)
- Consistent with lymphocytic (Hashimoto) thyroiditis in the proper clinical context
- Consistent with granulomatous (subacute) thyroiditis
- Other

意义不明的细胞非典型性病变或滤泡性病变AUS/FLUS： 5～15%，重复FNA

III. **Atypia of Undetermined Significance or Follicular Lesion of Undetermined Significance**

IV. **Follicular Neoplasm or Suspicious for a Follicular Neoplasm**
- Specify if Hürthle cell (oncocytic) type

滤泡性肿瘤或可疑滤泡性肿瘤FNS/SFN： 15%～30%，叶切除术

V. **Suspicious for Malignancy**
- Suspicious for papillary carcinoma
- Suspicious for medullary carcinoma
- Suspicious for metastatic carcinoma
- Suspicious for lymphoma
- Other

可疑恶性肿瘤： 60～75%，近乎全切或叶切除

VI. **Malignant** 恶性：97～99%
- Papillary thyroid carcinoma
- Poorly differentiated carcinoma
- Medullary thyroid carcinoma
- Undifferentiated (anaplastic) carcinoma
- Squamous cell carcinoma
- Carcinoma with mixed features (specify)
- Metastatic carcinoma
- Non-Hodgkin lymphoma
- Other

The Bethesda System for Reporting Thyroid Cytopathology

2009年公布的TBSRTC系统

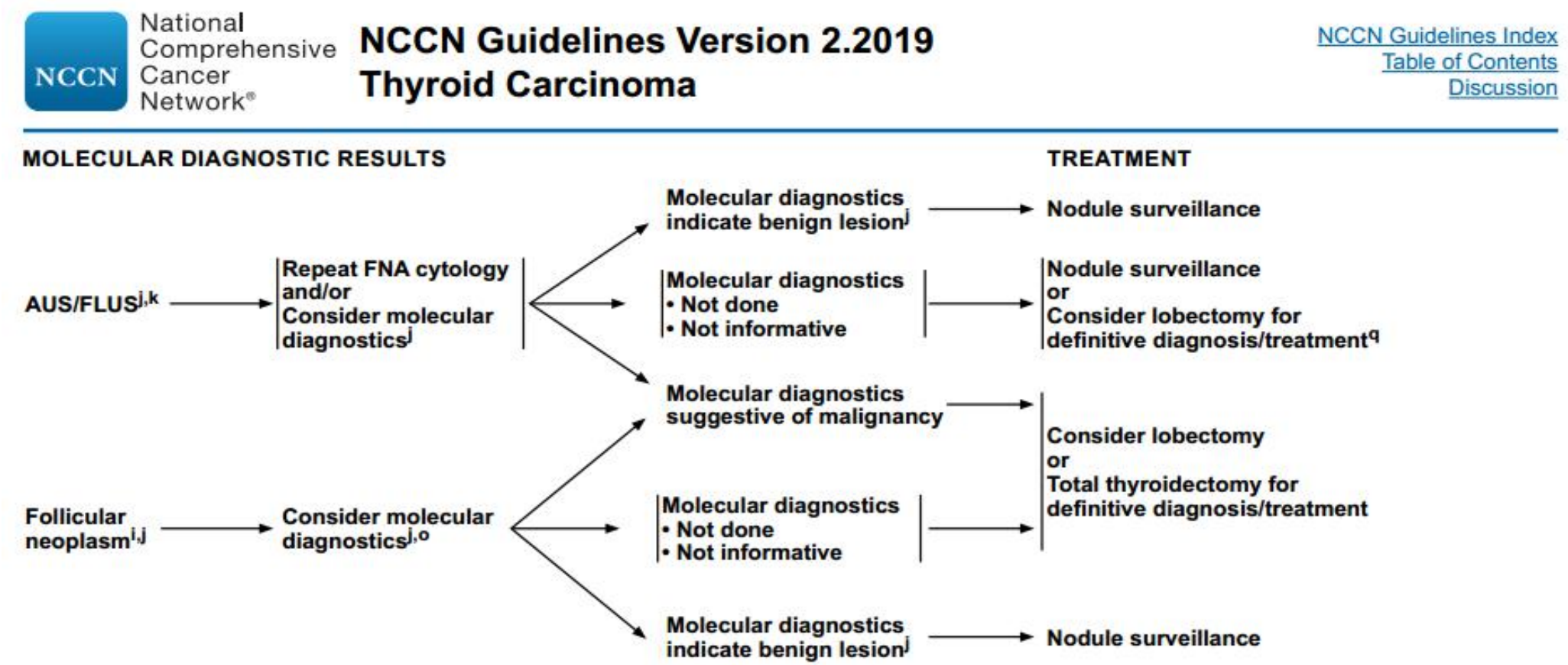
基于TBSRTC的恶性危险度分类

Table 7. The Bethesda system for reporting thyroid cytopathology: Diagnostic categories and risk of malignancy[1]

Diagnostic category	Estimated/predicted risk of malignancy by the Bethesda system (%)[1]	Actual risk of malignancy in nodules surgically excised (%, median (range))[2]
Nondiagnostic or Unsatisfactory	1-4	20 (9-32)
Benign	0-3	2.5 (1-10)
Atypia of Undetermined Significance or Follicular Lesion of Undetermined Significance (AUS/FLUS)	5-15	14 (6-48)
Follicular Neoplasm or Suspicious for a Follicular Neoplasm (FN/SFN)	15-30	25 (14-34)
Suspicious for Malignancy (SUSP)	60-75	70 (53-97)
Malignant	97-99	99 (94-100)

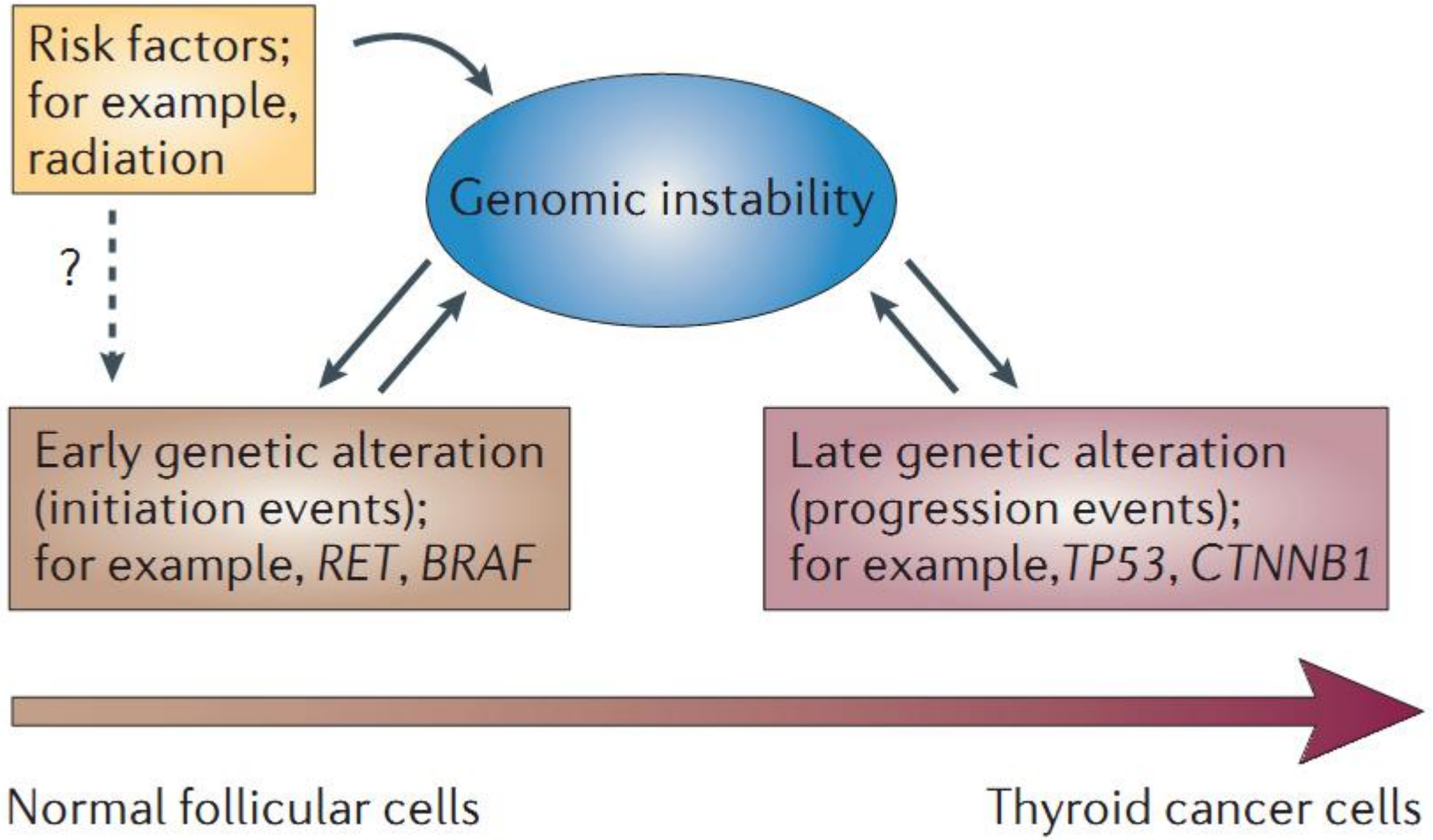
[1]As reported in The Bethesda System by Ali &Cibas, 2009 (1076)
[2]Based on the meta-analysis of 8 studies reported by Bongiovanni et al. (103). The risk was calculated based on the portion of nodules in each diagnostic category that underwent surgical excision and likely is not representative of the entire population, particularly of non-diagnostic and benign diagnostic categories.

2019 NCCN指南



对FNA仍不能确定良恶性的甲状腺结节，对穿刺标本进行分子标记物的检测

甲状腺肿瘤多步骤演变模型



Pathogenetic mechanisms in thyroid follicular-cell neoplasia

甲状腺肿瘤分子诊断标记物

- 基因突变
- 染色体重排
- 基因表达分类谱gene expression classifier (Afirma)
- IHC： galectin-3

评价指标：

1. 敏感性、特异性、阳性预测率（PPV）及阴性预测率（NPV）
2. 对标本的要求
3. 检测可行性
4. 临床接受度

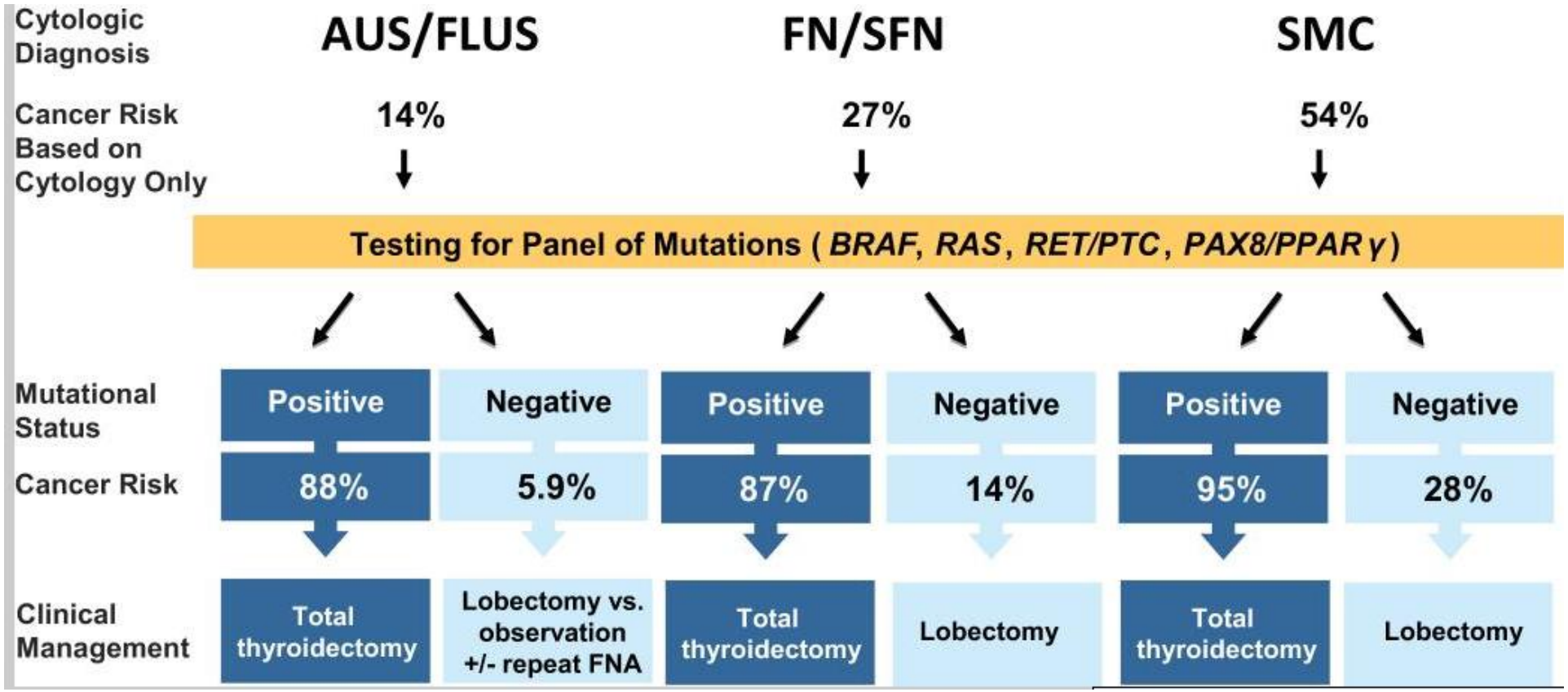
7-gene test

Genes (corresponding amino acid changes or fusion transcripts)
BRAF (p.V600E)
HRAS (p.G12V, p.Q61K, p.Q61R)
KRAS (p.G12A, p.G12C, p.G12D, p.G12R, p.G12S, p.G12V, p.G13D)
NRAS (p.Q61K, p.Q61L, p.Q61R)
PAX8-PPARG (PAX8-PPARG[a])
RET-CCDC6 (RET-PTC1)
RET-NCOA4 (RET-PTC3)

	AUS/FLUS	FN/SFN	SUSP
敏感性	63	57	68
特异性	99	97	96
PPV	88	87	95
NPV	94	86	72

Cancer probability in thyroid nodules with indeterminate cytology based on specific cytological diagnosis and results of molecular testing performed in FNA

	Cancer risk			
	AUS/FLUS cytology (n = 247)	FN/SFN cytology (n = 214)	SMC cytology (n = 52)	All indeterminate cytology (n = 513)
Cytology only	14%	27%	54%	24%
Any mutation identified	88%	87%	95%	89%
RAS	84%	85%	88%	85%
BRAF	100%	100%	100%	100%
PAX8/PPARγ	100%	100%	100%	100%
RET/PTC	na	na	100%	100%
No mutation identified	5.9%	14%	28%	11%


Cytologic Diagnosis
AUS/FLUS
FN/SFN
SMC
Cancer Risk Based on Cytology Only
14%
27%
54%
Testing for Panel of Mutations (BRAF, RAS, RET/PTC, PAX8/PPARγ)
Mutational Status
Positive
Negative
Positive
Negative
Positive
Negative
Cancer Risk
88%
5.9%
87%
14%
95%
28%
Clinical Management
Total thyroidectomy
Lobectomy vs. observation +/- repeat FNA
Total thyroidectomy
Lobectomy
Total thyroidectomy
Lobectomy

基因表达分类谱
（Gene expression classifier, GEC）

ORIGINAL ARTICLE

Endocrine Care

Molecular Classification of Thyroid Nodules Using High-Dimensionality Genomic Data

- mRNA表达分析：Human Exon 1.0 ST arrays (Affymetrix)
- 在315甲状腺结节中检测247,186转录体
- NPV=96%，特异性=84%
- 临床应用价值：降低真正良性结节的甲状腺腺叶切除术

J Clin Endocrinol Metab, December 2010, 95(12):5296–5304

基因表达分类谱
（Gene expression classifier, GEC）

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Preoperative Diagnosis of Benign Thyroid Nodules with Indeterminate Cytology

METHODS

We performed a 19-month, prospective, multicenter validation study involving 49 clinical sites, 3789 patients, and 4812 fine-needle aspirates from thyroid nodules 1 cm or larger that required evaluation. We obtained 577 cytologically indeterminate aspirates, 413 of which had corresponding histopathological specimens from excised lesions. Results of a central, blinded histopathological review served as the reference standard. After inclusion criteria were met, a gene-expression classifier was used to test 265 indeterminate nodules in this analysis, and its performance was assessed.

RESULTS

Of the 265 indeterminate nodules, 85 were malignant. The gene-expression classifier correctly identified 78 of the 85 nodules as suspicious (92% sensitivity; 95% confidence interval [CI], 84 to 97), with a specificity of 52% (95% CI, 44 to 59). The negative predictive values for "atypia (or follicular lesion) of undetermined clinical significance," "follicular neoplasm or lesion suspicious for follicular neoplasm," or "suspicious cytologic findings" were 95%, 94%, and 85%, respectively. Analysis of 7 aspirates with false negative results revealed that 6 had a paucity of thyroid follicular cells, suggesting insufficient sampling of the nodule.

CONCLUSIONS

These data suggest consideration of a more conservative approach for most patients with thyroid nodules that are cytologically indeterminate on fine-needle aspiration and benign according to gene-expression classifier results. (Funded by Veracyte.)

- 167 个基因表达
- 49家医院、3700患者、4800个FNA

	总	AUS/FLUS	FN/SFN	SUSP	良性	恶性
敏感性	92	90	90	94	100	100
特异性	52	53	49	52	70	100
PPV	47	38	37	76		
NPV	93	95	94	85		

特异性低，而NPV高
排除性的指标

NEJM, 2012, 8(23):705~715

ThyroSeq NGS panel基因列表

共涉及14个基因的突变及7个基因的42个融合型别

点突变/缺失	基因融合
AKT1	RET
BRAF	PPARG
CTNNB1	BRAF
GNAS	NTRK1
HRAS	NTRK3
KRAS	ALK
NRAS	THADA
PIK3CA	
PTEN	
RET	
TERT	
TP53	
TSHR	
EIF1AX	

	FN/SFN	AUS/FLUS
敏感性	90	91
特异性	93	92
PPV	83	77
NPV	96	97

ThyroSeq multi-gene NGS panel 对FN/SFN及AUS/FLUS细胞学癌的诊断检测具有高的敏感性及特异性。

匹兹堡大学Nikiforova团队应用

Cancer. 2014;120(23):3627-34
THYROID, 2015; 25 (11): 1217~1235

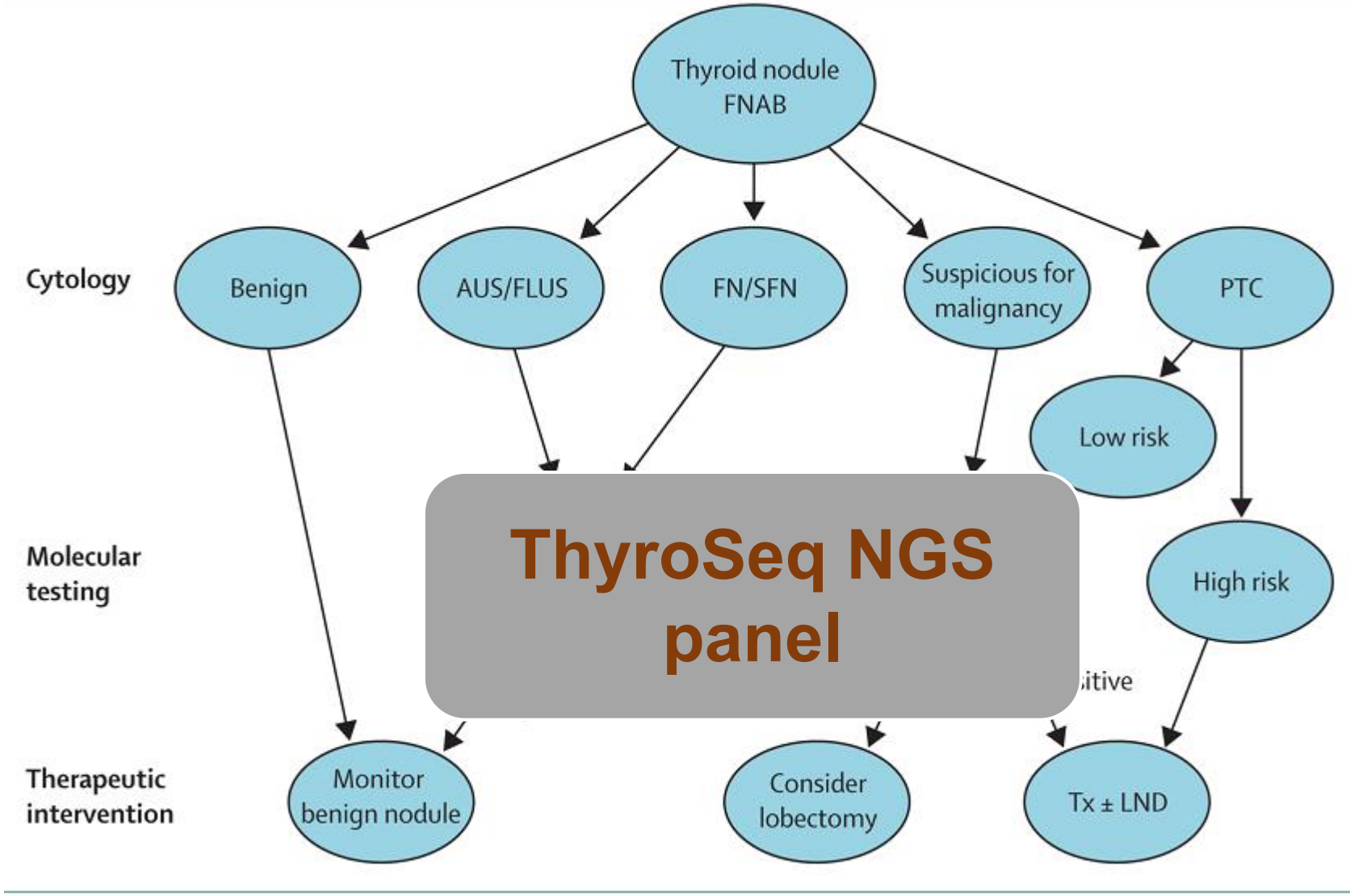
分子检测方法的综合比较

	GEC	7-gene panel	TyroSeq v2
敏感性	80%	57~75%	90%
特异性	50%	97~100%	93%
阳性预测值	15~37%	87~100%	83%
阴性预测率	75~94%	79~86%	95%

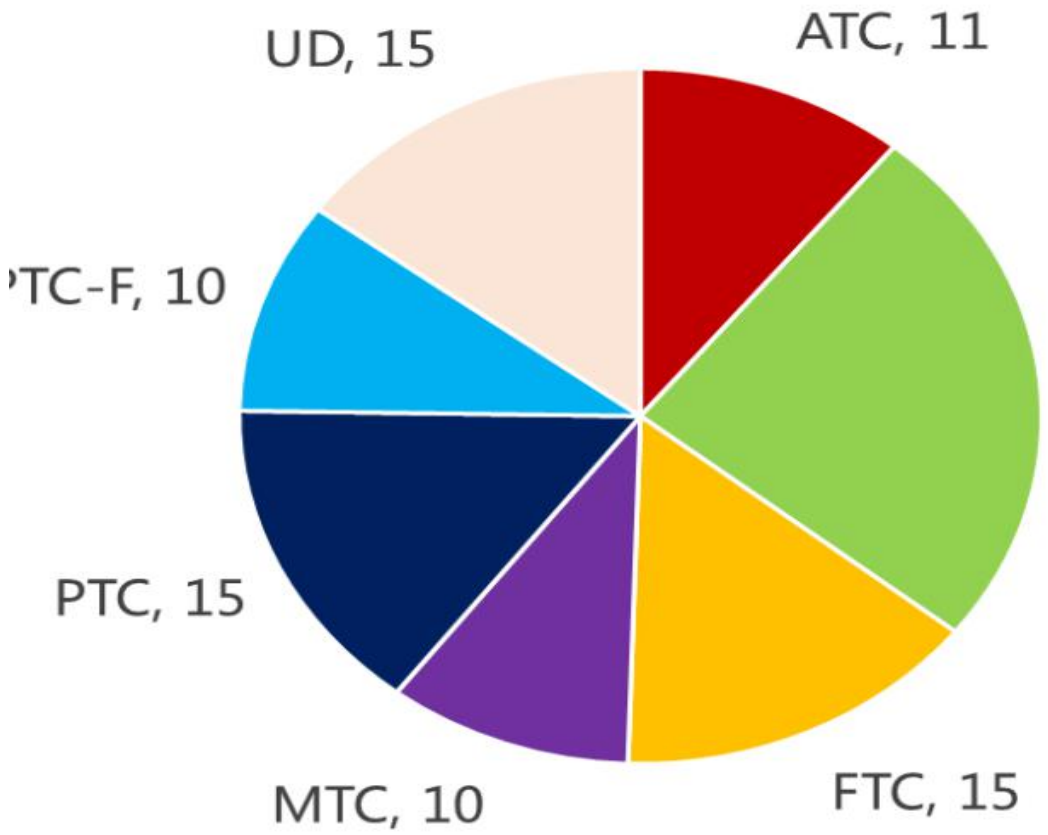
排除性
rule out检测

确诊性
Rule in检测

基于FNAB及分子标记物检测的甲状腺结节管理模式图



样本选择



ATC:	未分化甲状腺癌
FTC:	滤泡状甲状腺癌
PTC:	乳头状甲状腺癌
PTC-F:	滤泡型乳头状癌
MTC:	甲状腺髓样癌
Benign:	良性
	毒性甲状腺肿
	结节性甲状腺肿
	桥本甲状腺炎
UD:	难于分型

节选自华西医院刘卫平

依据分子分型进行良恶性预测

如果依照基于DNA检测的分子分型对甲状腺组织进行良恶性预测：

	实际为恶性	实际为良性
预测为恶性	52	5
预测为良性	9	20

- ✓ 敏感性 (Sensitivity) = 85.2%
- ✓ 阳性预测值 (PPV) = 91.2%

与天津肿瘤医院65例甲状腺癌赛甄验证实验结果相似：

- ✓ 敏感性 (Sensitivity) = 81.5%
- ✓ 阳性预测值 (PPV) = 95.7%

节选自华西医院刘卫平

依据分子分型进行良恶性预测

如果依照基于DNA检测的分子分型对甲状腺组织进行良恶性预测：

	实际为恶性	实际为良性
预测为恶性	52	5
预测为良性	9	20

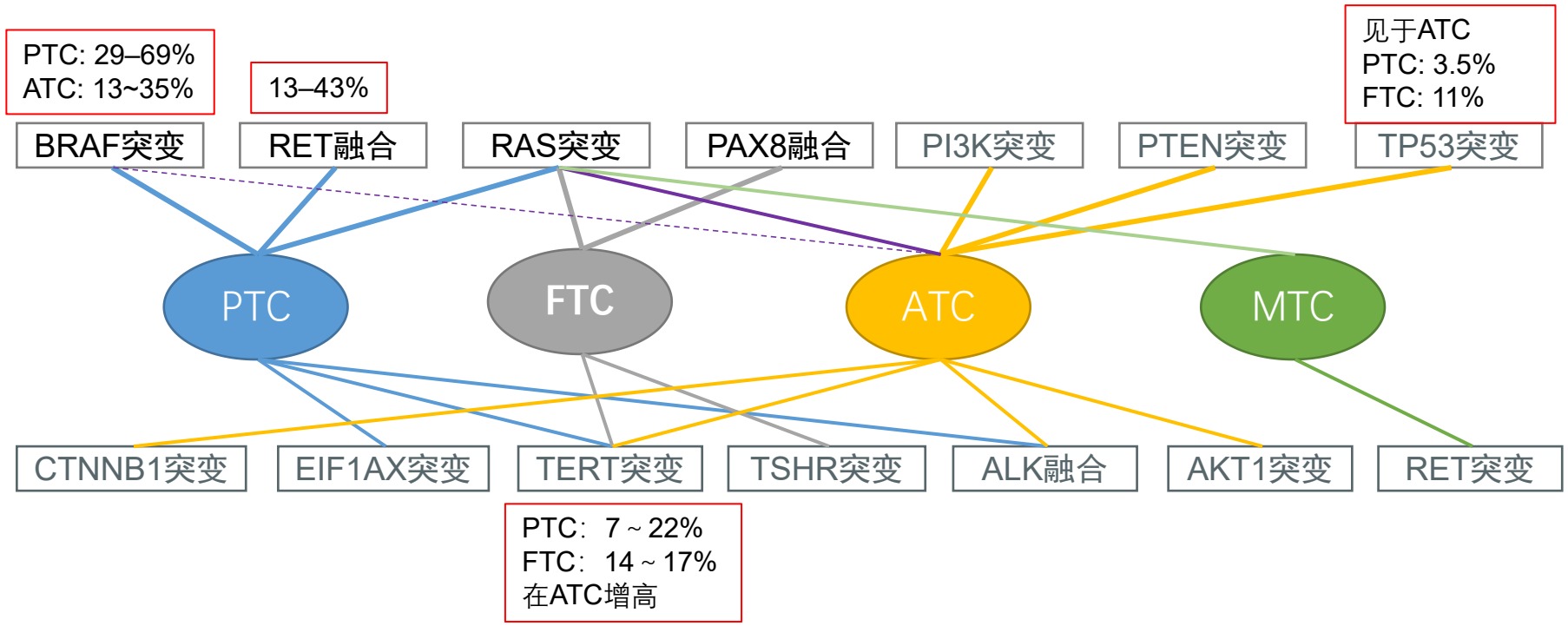
✓ Sensitivity = 85.2%

- ATC: 10 out of 11 = 91%
- FTC: 15 out of 15 = 100%
- MTC: 8 out of 10 = 80%
- PTC: 13 out of 15 = 87%

} 90.2%

- PTC-F:= 60% 6 out of 10

对于滤泡型乳头状癌以外的病理亚型敏感性>90%



高侵袭性甲状腺癌可能与

（1） BRAF V600E联合其他致癌基因的突变；

（2） TERT启动子突变 （独立或与BRAF基因突变共存）；

（3） TP53突变

甲状腺癌基因突变不同比例分布

Oncogene	Mean prevalence (%)
BRAF	48
RET/PTC	20
APC	15
SMAD4	14
CTNNB1	12
IDH1	10
NTRK1	10
CDKN2A	9
EGFR	8
NRAS	4
HRAS	2
KRAS	2
PIK3CA	2

Data derived from:
http://www.sanger.ac.uk/genetics/CGP/cosmic

- 位于7q34，丝/苏氨酸特异性激酶
- 突变类型：90% exon15 T1799A V600E；10% exon11 甘氨酸环
- 突变发生肿瘤： 结直肠癌：5%~10%；恶性黑色素瘤：25%；
  甲状腺乳头状癌：30%

- 1987年, 发现a new oncogene
- 1997年，RET重排，命名为RET/PTC， 激活RET-酪氨酸激酶，启动MAPK
- 2012年, 发现13种RET重排亚型

甲状腺癌遗传性变异

Table 2 | **Genetic defects in thyroid cancer**

Genetic alteration	Well-differentiated thyroid carcinoma		Poorly differentiated thyroid carcinoma	Undifferentiated thyroid carcinoma	Post-Chernobyl childhood thyroid cancer
	Papillary thyroid carcinoma	Follicular thyroid carcinoma			
RET rearrangement	13–43%	0%	0–13%	0%	50–90%
BRAF mutation	29–69%	0%	0–13%	10–35%	0–12%
BRAF rearrangement	1%	Unknown	Unknown	Unknown	11%
NTRK1 rearrang	5–13%	Unknown	Unknown	Unknown	3%
Ras mutation	0–21%	40–53%	18–27%	20–60%	0%
PPARG rearrangement	0%	25–63%	0%	0%	Unknown
CTNNB1 mutation	0%	0%	0–25%	66%	Unknown
TP53 mutation	0–5%	0–9%	17–38%	67–88%	Unknown

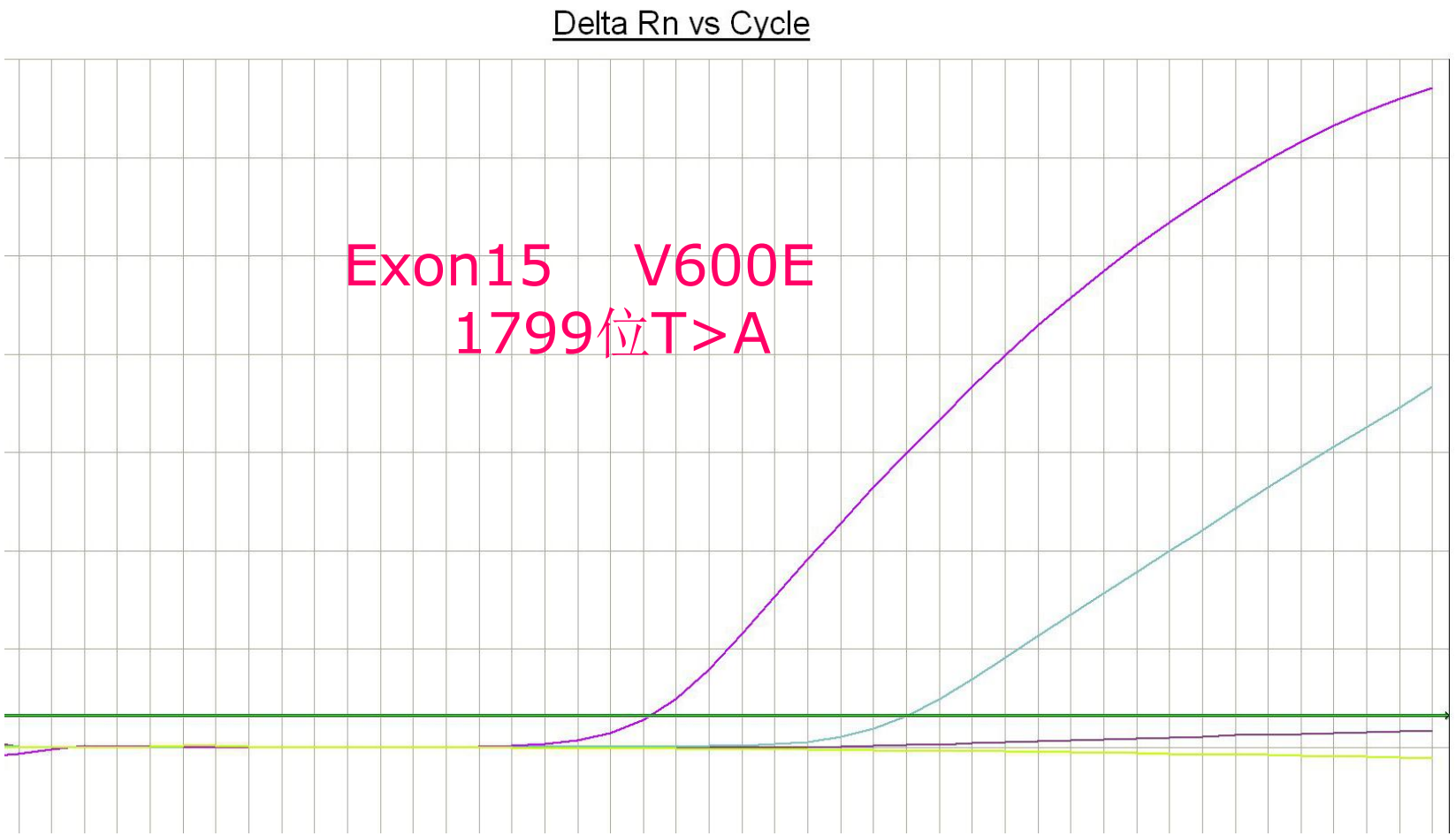
CTNNB1, β-catenin; NTRK1, neurotrophic tyrosine kinase receptor, type 1; PPARG, peroxisome-proliferator-activated-receptor-γ.

第一个在甲状腺癌中发现的激活的TK受体

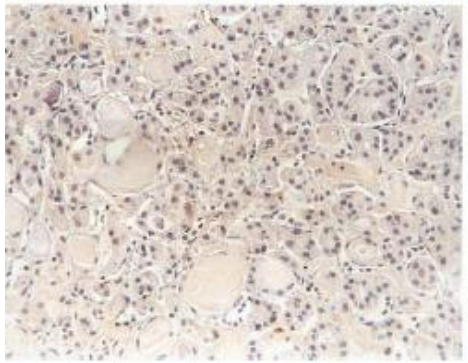
第二发现的重排

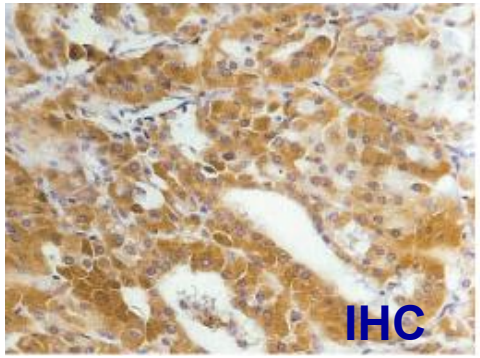
与其他肿瘤不同，Ras最少参与甲状腺癌的发生。HRAS及NRAS的codon61. 突变多发生于FTC及F-PTC，少经典的PTC.
有些争论，在良性肿瘤中出现RAS突变

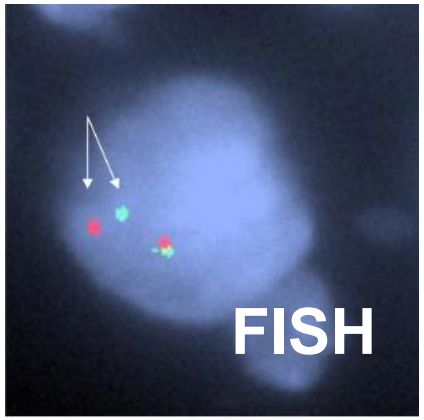
qPCR 法检测B-raf基因exon15突变

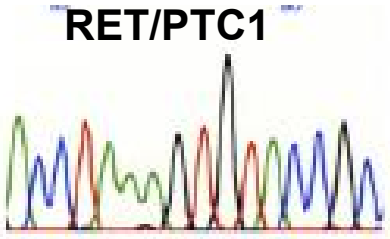


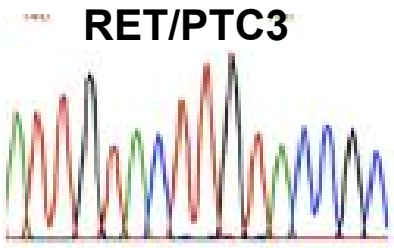
RET基因检测方法

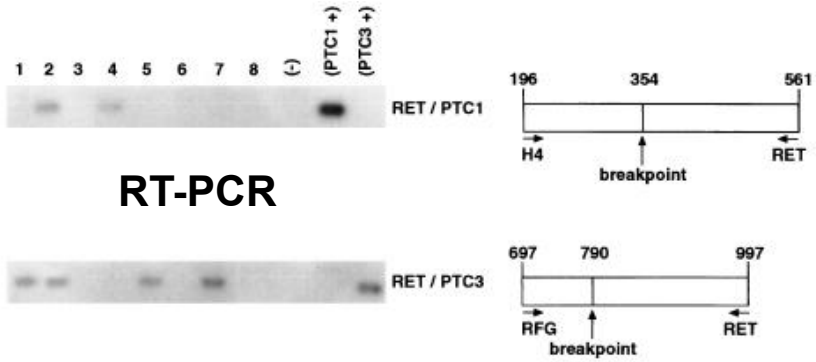



IHC


FISH

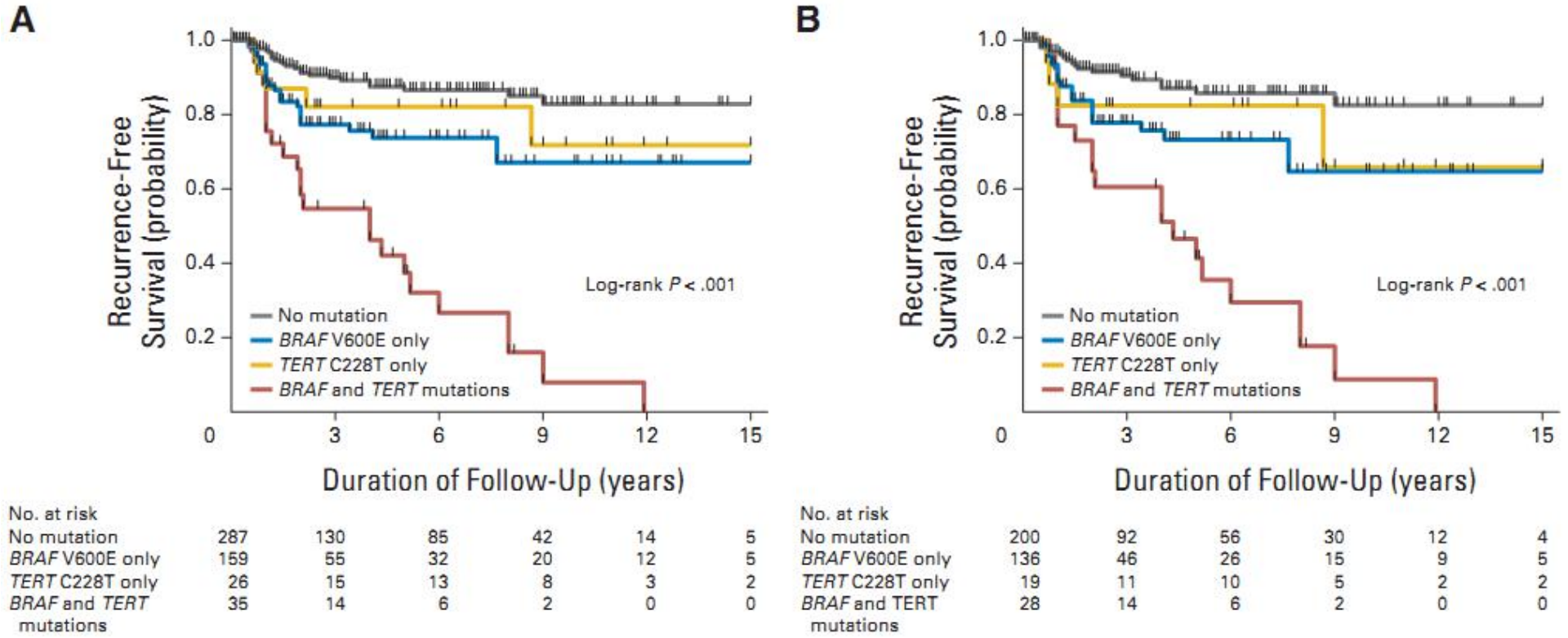

RET/PTC1


RET/PTC3


1 2 3 4 5 6 7 8 (-) (PTC1 +) (PTC3 +)
RET / PTC1
196 354 561
H4 breakpoint RET
RT-PCR
RET / PTC3
697 790 997
RFG breakpoint RET

BRAF V600E and TERT promoter C228T突变联合检测的预后价值

507 patients (365 women and 142 men) age 45.9 ± 14.0 years (mean ± SD) who were treated for PTC with total thyroidectomy and clinically observed between 1990 and 2012 at Johns Hopkins Hospital



(A) Results of the analyses of patients with PTC of all types. (B) Results of the analyses of conventional variant PTC only.

J Clin Oncol 32:2718-2726

小结

2015 年ATA 指南推荐对于FNA 诊断为不能确定性质的结节，应依据临床风险因素、超声特征、病人的选择与可能获得的基因突变分子检测结果选择治疗方式，医生在临床决策中应告知病人可有的选择及可行性。

基因突变分子检测的推荐级别较低，技术方法正在进一步**探索**，可作为FNA的有益有效补充

分子标记物对甲状腺癌辅助分类、预后的预测争议颇多

+

基于传导通路关键基因的靶向药物

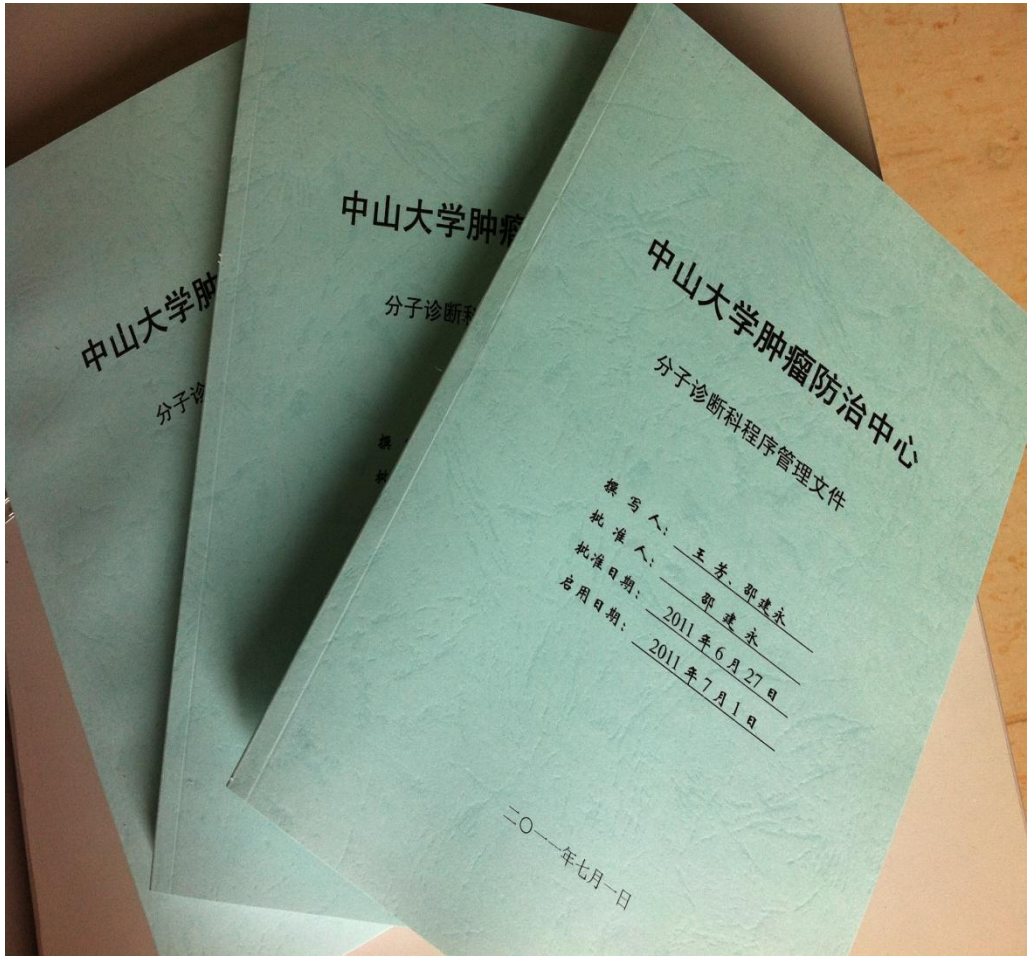
多基因的平行检测势在必行

进一步的积累数据，洞悉现在，发现未来

如何实现精准的分子诊断？

——建立分子检测标准操作程序（SOP）

- 建立严格的质量控制体系，保证结果的准确性；
- 完成实验室20余项分子检测项目的SOP撰写。



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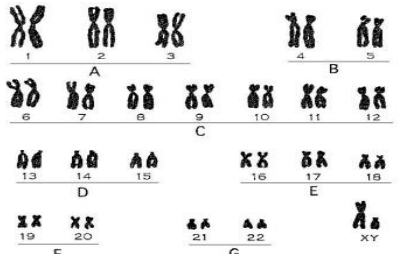
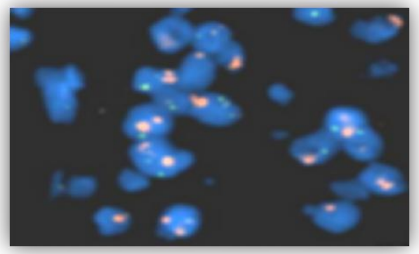
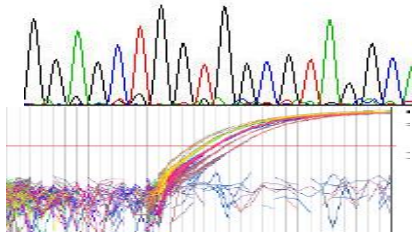
现有的完备的技术体系



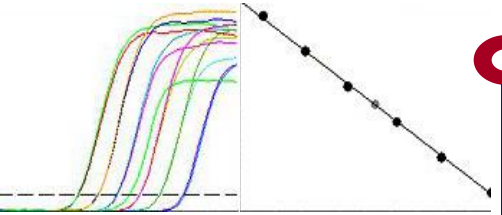
核型



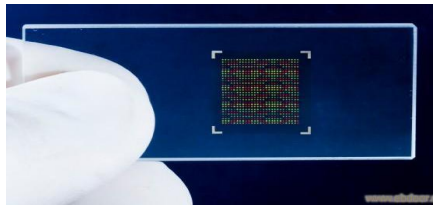
荧光原位杂交



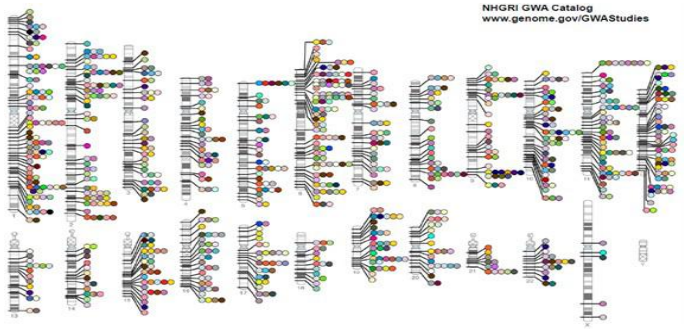
单基因突变



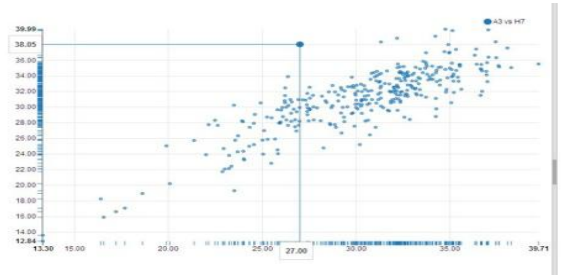
病原体含量



基于芯片技术的高通量多基因分型



高通量测序



数字PCR

多层次检测平台的综合应用

瘤种	检测基因	检测技术
胶质瘤	1p/19q、MGMT、EGFR、H3F3A K27M	FISH、qPCR
	IDH1、IDH2、EGFRvIII、TERT、KIAA1549-BRAF	测序、qPCR
鼻咽癌	EBV、易感性芯片筛查	qPCR、芯片
甲状腺癌	BRAF、RET	qPCR
肺癌	EGFR、KRAS、BRAF、HER2、NRAS、BIM	qPCR
	ALK、MET、ROS1、RET	FISH
	IGF1R、FGFR1	FISH
乳腺癌	遗传性乳腺癌/卵巢癌筛查	NGS
	HER2、PIK3CA、Topo II α	FISH、测序
胃癌	HER2、MET、FGFR2，遗传性胃癌	FISH、NGS
肠癌	遗传性大肠癌筛查，MSI	NGS、基因片段分析
	靶向药物筛选：RAS、BRAF、PIK3CA、PTEN	qPCR、测序、FISH、芯片
GIST	KIT、PDGFRA、SHDB	测序
恶性黑色素瘤	BRAF、KIT、PDGFRA；辅助诊断	qPCR、测序、FISH、芯片
尿路上皮癌	chr3、7、17，p16	FISH
神经母细胞瘤	NMYC、ALK	FISH、测序
软组织肿瘤	SS18、EWSR1、DDIT3、MDM2、FOXO1、USP6	FISH
淋巴瘤	Ig、TCR基因重排	基因片段分析
	IGH/BCL2、IGH/CCND1、IGH/MYC、API2/MALT1、IGH/MALT1	FISH
	IGH、MYC、BCL6、ALK	
血液肿瘤	P53/1q21/D13S319/RB1、IGH/FGFR3、IGH/MAF	FISH
	p53/ATM/CSP12/Rb1/D13S25	FISH
	AML1/ETO、PML/RARA、CBFB、MLL、JAK2	FISH、qPCR、测序
	NPMI、FLT3-ITD、CEBPA、CKIT	
	TEL/AML1、MLL、BCR/ABI1	FISH、qPCR
	NOTCH1、FBXW7	测序
	5/5q、7/7q、20q、8、Y	FISH
病原体	EBV、HBV、HCV、HPV、TB	qPCR
化疗药物	UGT1A1、CYP2D6、MTHFR、GSTP1	测序
NGS项目	遗传性乳腺癌/卵巢癌、肠癌、胃癌等14个遗传性肿瘤	
	ctDNA基因突变	
	泛癌肿基因变异	

16个瘤种、102个项目

遗传诊断

早期筛查

辅助诊断

化疗预测

靶向筛选

预后分层

洞悉现在　发现未来

谢 谢