Thyroid FNA
Ancillary Studies

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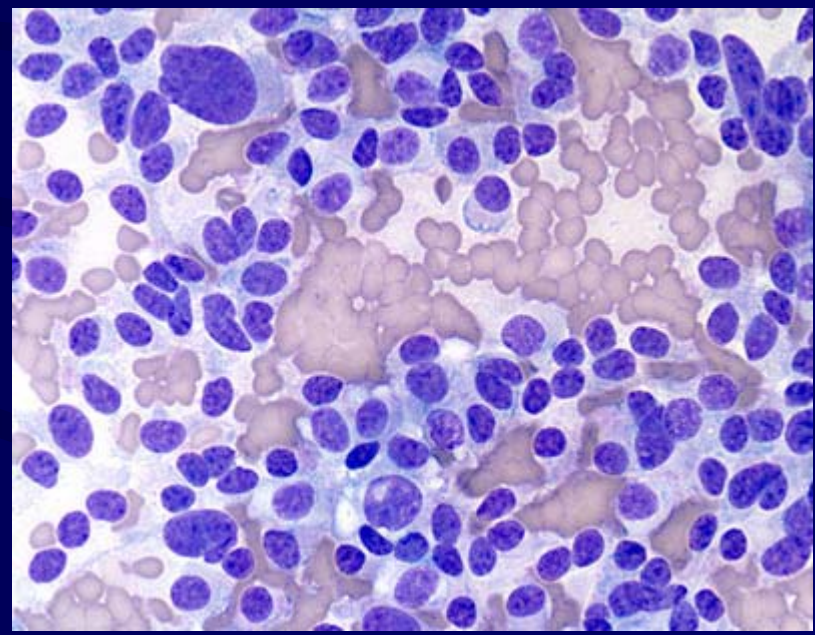
What are the goals of ancillary studies of thyroid FNAs?

- Characterization of a known malignancy (<1%)
  - Suspected medullary carcinoma, lymphoma or metastasis

- Increased specificity for malignancy (>10%)
  - Proteins (IHC)
  - Nucleic acids (mutations and translocations)

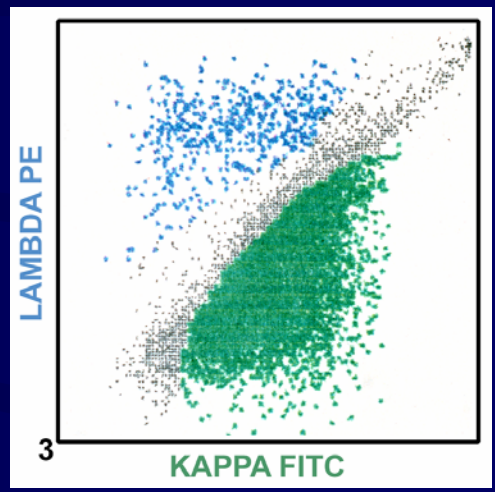
Medullary Carcinoma

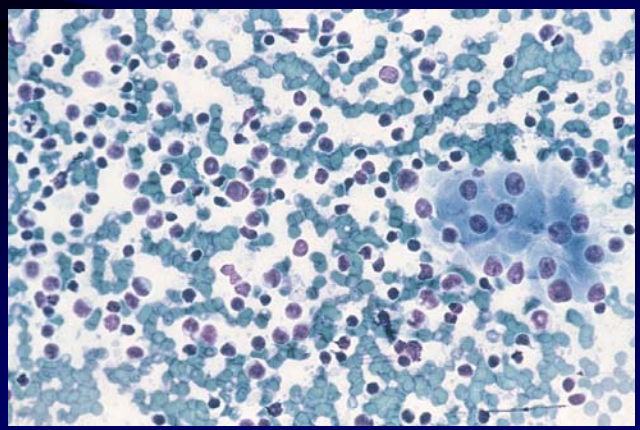
- IHC
  - Calcitonin (100%)
  - Chromogranin (100%)
  - CEA (94%)
  - Thyroglobulin (6%)
- Serum calcitonin

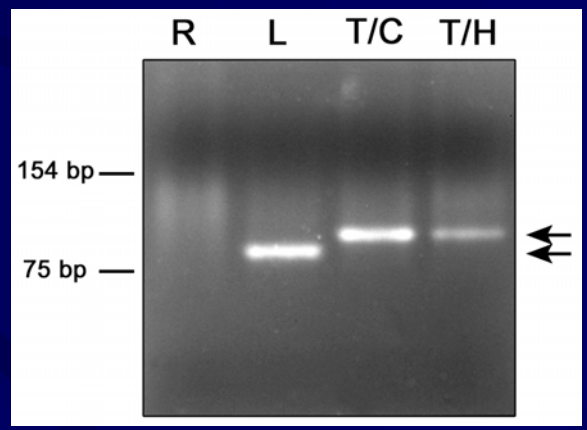


Thyroid Lymphoma

- Flow cytometric immunophenotyping
  - IHC
  - (FISH, IgG gene rearrangement)

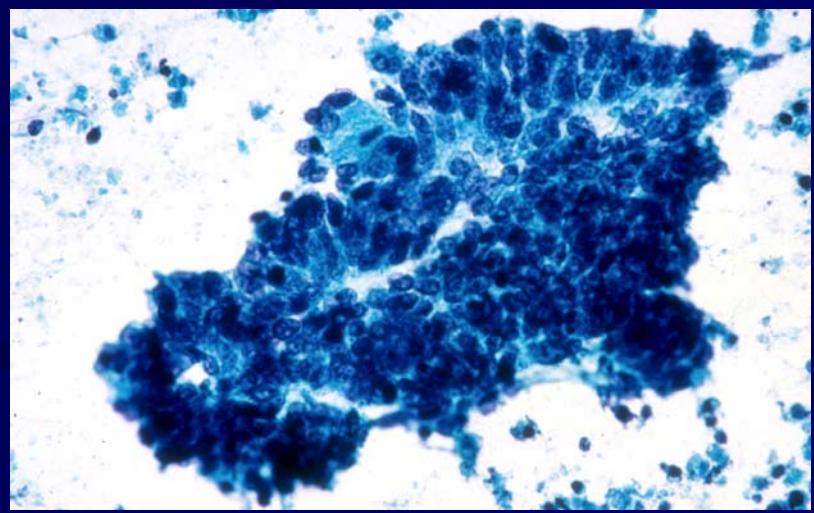






Metastasis

- IHC (TTF-1, etc.)
  - Renal cell carcinoma
  - Breast carcinoma
  - Lung carcinoma
    (TTF-1 +)
  - Colorectal carcinoma
  - Melanoma
  - Lymphoma



Clark, D.P. and Faquin, W.C., *Thyroid Cytopathology*, 2005, Springer

What are the goals of ancillary studies of thyroid FNAs?

- Characterization of a known malignancy
  - Suspected medullary carcinoma
  - Suspected lymphoma
  - Suspected metastasis
- Increased specificity for malignancy
  - Proteins (IHC)
  - Nucleic acids (mutations and translocations)

Accuracy of Thyroid FNA

• Accuracy	>95%
• False-negative	1-11%
• False-positive	0-7%
• Positive predictive value	89-98%
• Negative predictive value	94-99%
• Sensitivity	43-98%
• Specificity	72-100%

Gharib H, Goellner JR. Ann Intern Med. 1993 Feb 15;118(4):282-9.

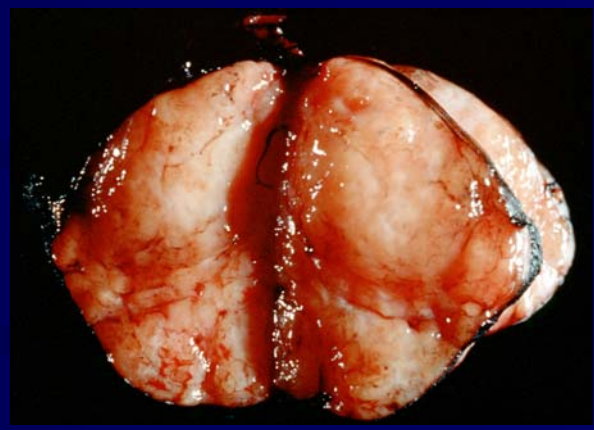
Thyroid FNA
False-negatives

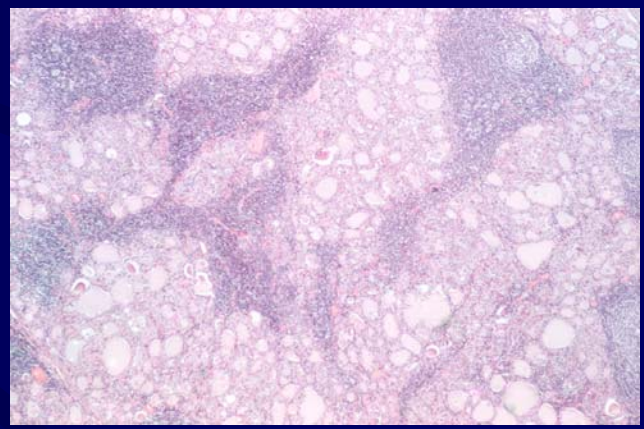
- Uncommon
- True false-negative rate difficult to determine due to lack of surgical follow-up.
- Interpretive error. Uncommon.
  - Adequacy.
- Sampling error.
  - Effect of US guidance?

- Amrikachi M, et al. Arch Pathol Lab Med. 2001 Apr;125(4):484-8.
- Ylagan LR, et al.. Thyroid. 2004 Jan;14(1):35-41.

Thyroid FNA
False-positives

- Uncommon
- Common cause: Hashimoto's thyroiditis
  - Cytology: Papillary thyroid carcinoma or Follicular neoplasm





- Amrikachi M, et al. Arch Pathol Lab Med. 2001 Apr;125(4):484-8.
- Ylagan LR, et al.. Thyroid. 2004 Jan;14(1):35-41.

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The Indeterminate/Suspicious Thyroid FNA

- Common
  - Approx. 10-15% of FNAs
- Morphologically and biologically diverse
  - Follicular neoplasm, Suspicious for PTC, etc.
- Surgical outcome?
  - Most are benign (up to 85%)

- Ravetto C, et al. Cancer. 2000 Dec 25;90(6):357-63
- Gharib H, et al. Clin Lab Med. 1993 Sep;13(3):699-709. .
- Castro MR, Gharib H. Ann Intern Med. 2005 Jun 7;142(11):926-31.
- Segev, et al. Acta Cytol 2003; 47:709-722

Increased specificity for malignancy

- Minimize surgery for benign disease
- Minimize inadequate treatment of malignancy.
  - Second-stage completion thyroidectomy
- Goal: remove only clinically-relevant malignant or pre-malignant lesions
- Currently an “ASC-US-like” situation using Suspicious category to increase sensitivity, at cost of specificity.

Malignancy-associated proteins: IHC

- Galectin 3
- CK-19
- HBME-1

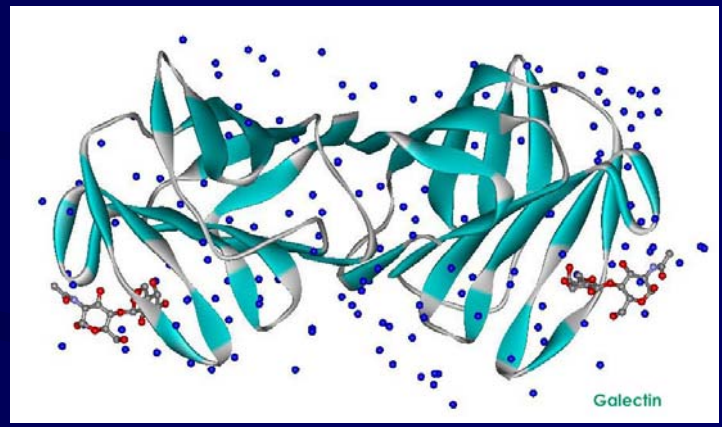
Thyroid Immunohistochemistry

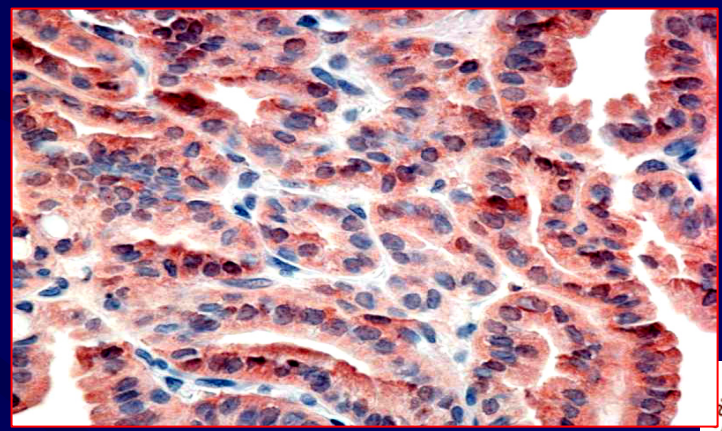
- Potential Problems with IHC analysis:
  - Variable fixation
  - Variable methods for antigen retrieval and staining
  - Inadequate antibody validation and lot-to-lot variability
  - Variable oxidation after sectioning
- Important to distinguish results from FNAs vs. whole tissue samples.
- False-positive results due to endogenous thyroid biotin when using Avidin-Biotin Complex or the Strepavidin-Biotin methods.

- Bast RC Jr, et al. Clin Cancer Res. 2005 Sep 1;11(17):6103-8.
- Kashima K, et al. Mod Pathol 1997; May;10(5):515-9.

Galectin-3

- Carbohydrate-binding protein
  - implicated in cell growth, adhesion, differentiation, and tumor progression
  - Highly expressed in malignant lesions (92%)
  - Rarely expressed in benign lesions (14%)





- Coli, A , et al. Histopathology. 2002 Jan;40(1):80-7.
- Herrmann ME, et al. Arch Pathol Lab Med. 2002 Jun;126(6):710-3.
- Prof. Dr. H.-J. Gabius www.lectins.de/Galectin.jpg

Galectin-3 Detection in FNAs

- "This diagnostic test method, which consistently improves the accuracy of conventional cytology, has been recently validated in a large international multicenter study and is going to impact hardly [sic] the clinical management of patients bearing thyroid nodular diseases."
  - Retrospective: n=165, 50% malignant; Prospective: n=226
  - Cell blocks; Ab dilution of "1:200 to 1:500"
  - Gasbarri A, et al.Biomed Pharmacother. 2004 Jul-Aug;58(6-7):356-9.
  - Bartolazzi A, et al. Lancet. 2001 May 26;357(9269):1644-50.

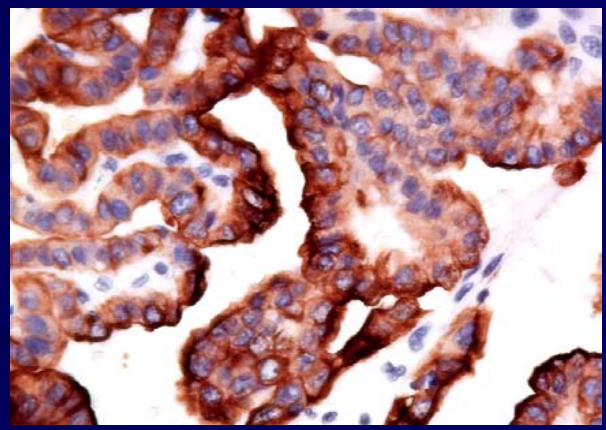
- "Galectin-3 immunohistochemistry does not appear to be a useful adjunct to diagnosis in thyroid FNA"
  - Cell blocks; no Ab dilution given
  - Mills LJ, et al. 2005 Jun;16(3):132-8.

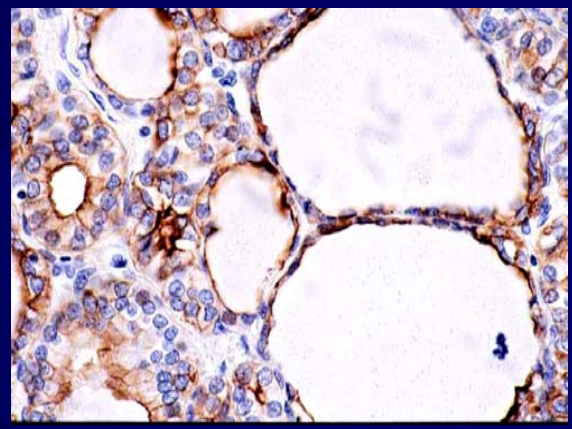
- "galectin-3 constitutes a useful marker in the diagnosis of thyroid lesions classified as undeterminate by conventional cytology"
  - Direct smears; Ab dilution of 1:100
  - Collet JF, et al. Br J Cancer. 2005 Nov 14;93(10):1175-81.

Cytokeratin 19

Low molecular weight intermediate filament protein

- Highly expressed in PTC (~85%)
- Can be expressed in benign nodules (~26%)
- Few FNA studies

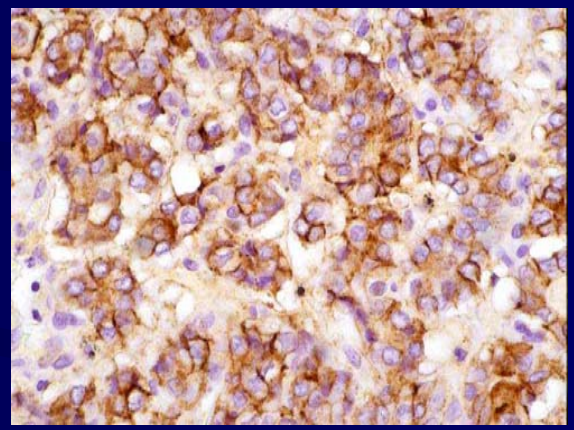


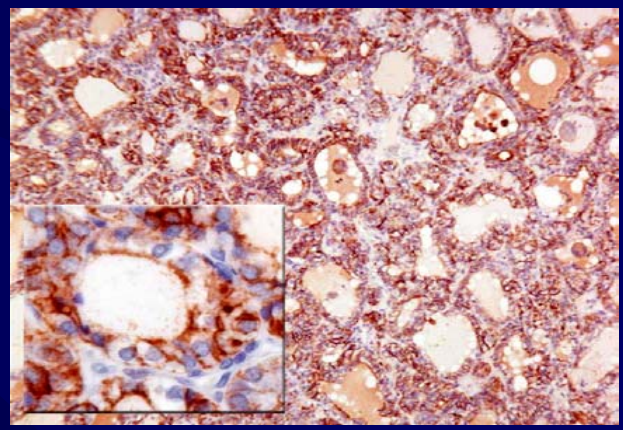


- Nasser SM, et al. Cancer. 2000 Oct 25;90(5):307-11.
- Segev, et al. Acta Cytol 2003; 47:709-722

HBME-1 Antigen

- Monoclonal antibody that reacts with an unknown antigen on microvilli of mesothelioma cells
  - Highly expressed in malignant lesions (~76%)
  - Less often expressed in benign lesions (~29%)
  - Few studies of FNAs





- Segev, et al. Acta Cytol 2003; 47:709-722

Other Protein Markers in the Diagnosis of Thyroid Tumors

- RET
- PPARG
- Thyroid Peroxidase
- BCL-2
- P53
- CD40
- Blood-group antigens
- S100
- Telomerase
- Lactoferrin
- CD44
- DAP4
- CD57
- CD-15

  – Segev, et al. Acta Cytol 2003; 47:709-722

PTC Diagnosis: panels of protein markers

- Histology:
  - CK-19, Galectin-3, HBME-1
    - Casey MB, et al.Endocr Pathol. 2003 Spring;14(1):55-60.
  - HBME-1, cytokeratin 19 and galectin-3
    - de Matos PS, et al.Histopathology. 2005 Oct;47(4):391-401
  - CK-19, HBME-1, RET
    - Cheung CC, et al. Mod Pathol. 2001 Apr;14(4):338-42.
  - Galectin-3, fibronectin-1, CITED-1, HBME1 and cytokeratin-19
    - Prasad ML, et al.Mod Pathol. 2005 Jan;18(1):48-57.
- FNAs:
  - Galectin-3, HBME-1, thyroperoxidase, cytokeratin-19 and keratan-sulfate
    - Saggiorato E, et al. Endocr Relat Cancer. 2005 Jun;12(2):305-17.
  - RET, HBME-1, Galectin-3
    - Rossi ED, et al. Cancer. 2005 Apr 25;105(2):87-95.

Nucleic acids: mutations and translocations

- RET/PTC
- RAS
- BRAF

- PAX8/PPARG

Genetic Markers of Papillary Thyroid Carcinoma?

- Common theme: RET-RAS-BRAF-MAPK pathway activation.
  - 2/3 of PTC have mutations/translocations in RET/PTC, NTRK, RAS, or BRAF.
  - These genetic alterations may be mutually exclusive
    - Suggests a common pathway
    - Complementary biomarkers?

RET/PTC gene Rearrangements

- Rearrangements of RET gene lead to fusion of the RET TK domain to the 5´-terminal regions of heterologous genes (RET/PTC 1-10), generating chimeric molecules
- These rearrangements lead to RET expression and activation
- Most common RET/PTC 1 & 3

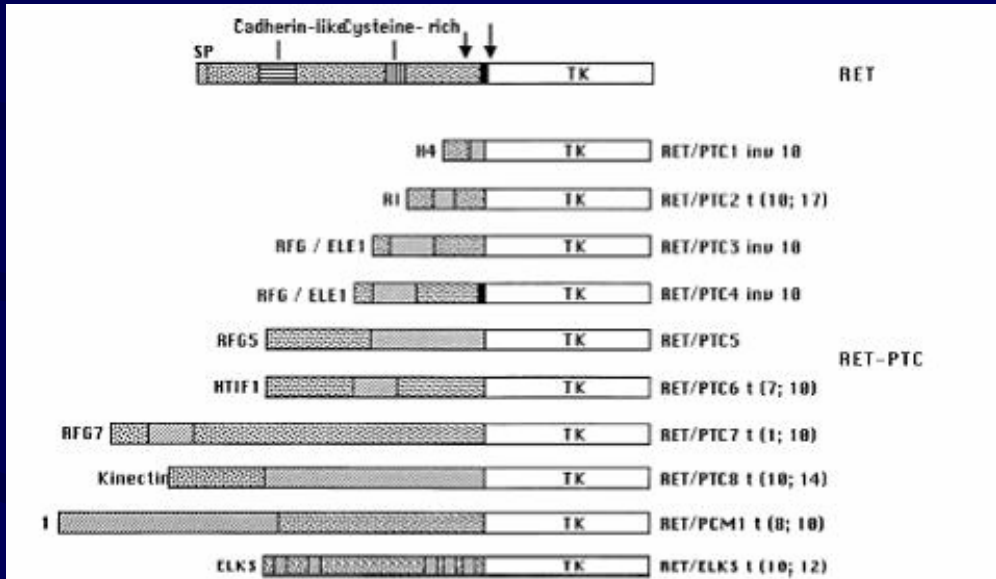


FIGURE 1. RET/PTC oncogenes, which are found in papillary thyroid carcinomas, are chimeric genes generated by the fusion of the RET tyrosine-kinase (TK)-encoding domain to different heterologous genes. *Arrows* indicate breakpoints in papillary thyroid cancer. Chromosomal aberrations leading to the formation of RET rearrangements are indicated. SP, signal peptide. Coiled-coild motifs in RERT fusion partners are indicated as *dashed boxes*.

Santoro M, et al.Ann N Y Acad Sci. 2002 Jun;963:11

RET/PTC gene Rearrangements

- Found in 2.5-40% of PTC
  - Detected prevalence is dependent on population and assay
- Particularly common in pediatric and radiation-induced PTC.
- Also found in Hurthle cell tumors and hyalinizing trabecular tumor
- Can be detected by RT-PCR in FNAs
  - Few studies

- *Fagin JA. Endocrinology. 2002 Jun;143(6):2025-8.*
- *Santoro M, et al.Ann N Y Acad Sci. 2002 Jun;963:116-21.*
- *Salvatore G, et al.J Clin Endocrinol Metab. 2004 Oct;89(10):5175-80.*
- *Domingues R, et al. Cytopathology. 2005 Feb;16(1):27-31.*
- *Cheung CC, et al. J Clin Endocrinol Metab. 2001 May;86(5):2187-90.*

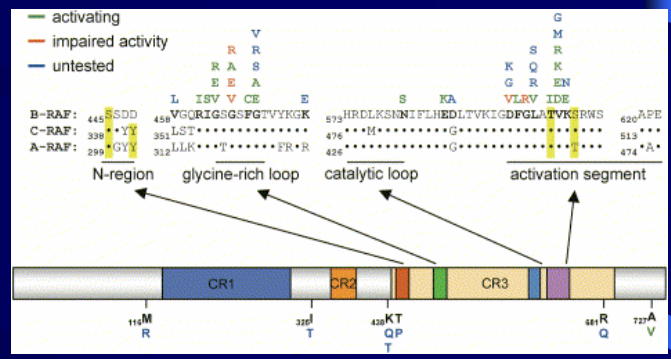
RAS mutations

- RAS
  - GTP-binding proteins whose activation via mutation result in deregulated cell cycle progression and uncontrolled cellular proliferation
  - Numerous mutations identified in HRAS, KRAS and NRAS
  - Mutations at codon 61 of N-RAS
    - 24% Follicular carcinomas
    - 5% PTC
    - 14% adenomas
    - 2.5% colloid nodules
  - Few applications to FNAs

- Vasko V, et al. J Clin Endocrinol Metab. 2003 Jun;88(6):2745-52.

BRAF mutations in papillary thyroid carcinoma

- Ser-Thr kinase in MEK signaling pathway
- Activating mutation
  - T⇒A substitution at nucleotide 1796 (V599E)
- Found in 66% of melanomas
  - Davies, et al. Nature 2003, 417:949-954
- Found in papillary thyroid carcinoma (44%)
  - Conventional PTC: 60 %
  - FVPTC: 12%
  - Tall cell: 77%
  - Anaplastic carcinoma (24%).
- Not identified in FTC, MTC, or benign nodules (n=542)
  - Review: Xing M. Endocr Relat Cancer. 2005 Jun;12(2):245-62.



Garnett MJ, Marais R.Cancer Cell. 2004 Oct;6(4):313-9.

BRAF mutations in thyroid FNAs

- 94% concordance with tissue results.
  - Cohen Y, et al. Clin Cancer Res. 2004 Apr 15;10(8):2761-5.
- Not detected in any benign FNAs (high specificity)
- Found in 44% of FNAs with PTC diagnosis
- Found in 17% of indeterminate/suspicious FNAs from PTC and 0% of indeterminate/suspicious FNAs from benign disease.
- Role in clinical management?

  - Xing M. Endocr Relat Cancer. 2005 Jun;12(2):245-62.

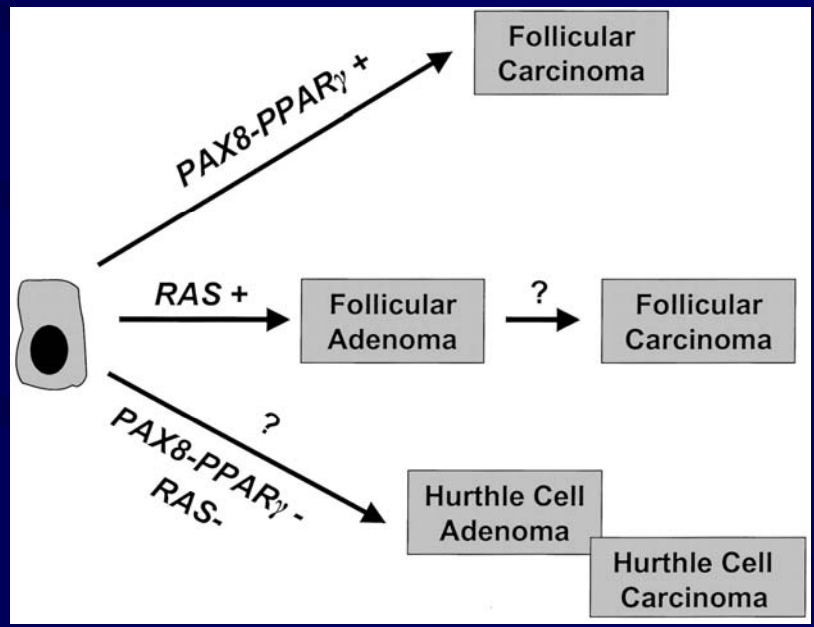
A Marker for Follicular Carcinoma? PAX8-PPARG Translocation

- Chromosomal Translocation [T(2;3)(q13;p25)] between a thyroid-specific transcription factor (PAX8) and a ubiquitous transcription factor (PPARG)
  - Produces a chimeric protein
  - Accelerates cell growth, reduces rates of apoptosis of thyroid cell lines
  - Inhibitor of the wild-type PPARG transcription factor.

- Kroll, et al., Science 289:1357, 2000

A Marker for Follicular Carcinoma? PAX8-PPARG Translocation

- Initial reports – Specific for follicular thyroid carcinoma
- Recent reports – Can be seen in benign lesions
  - RT-PCR
    - 35-56% Follicular thyroid carcinomas
    - 4-55% Follicular adenoma
      - Includes “tumors with partial, but not full, thickness capsular invasion”
    - Minimal application to FNAs.



Nikiforova MN, et al. J Clin Endocrinol Metab. 2003 May;88(5):2318-26.

Kroll, et al., Science 289:1357, 2000
Marques AR, et al. J Clin Endocrinol Metab. 2002 Aug;87(8):3947-52.
Cheung L, et, al. J Clin Endocrinol Metab. 2003 Jan;88(1):354-7.

Panels of Genetic Markers ?

- BRAF and RET/PTC
- Salvatore G, et al. J Clin Endocrinol Metab. 2004 Oct;89(10):5175-80.
- Soares P, et al.. Oncogene. 2003 Jul 17;22(29):4578-80.
- Domingues R, et al. Cytopathology. 2005 Feb;16(1):27-31.

- Gene expression profiles from microarrays?

Five Phases of biomarker development for early detection of cancer

- Phase 1: Preclinical Exploratory Studies
  - Promising directions identified.
- Phase 2: Clinical Assay Development and Validation
  - Clinical assay detects established disease
- Phase 3: Retrospective Longitudinal Repository Studies
  - Biomarker detects disease early
- Phase 4: Prospective Screening Studies
  - Characteristics of disease detected are identified
- Phase 5: Cancer Control Studies
  - Quantify impact of screening on population

•Pepe MS, et al. Phases of biomarker development for early detection of cancer. J Natl Cancer Inst. 2001 Jul 18;93(14):1054-61.

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Current status of biomarker development for thyroid carcinoma in FNAs?

- Phase 1: Preclinical Exploratory Studies
  - Several excellent candidate markers identified.
    - RET/PTC and PAX8/PPARg translocations, BRAF mutations
- Phase 2: Clinical Assay Development and Validation
  - Most assays not suitable for clinical laboratory
- Phase 3: Retrospective Longitudinal Repository Studies
  - Few examples of large, FNA-based studies
- Phase 4: Prospective Screening Studies
  - Virtually None
- Phase 5: Cancer Control Studies
  - None

Challenges

- Continue to identify candidate biomarkers
- Develop robust, reproducible assays that are appropriate for application to FNAs in the clinical lab
- Apply these assays (in combination) to large Retrospective Longitudinal Repository Studies and prospective screening studies.

Ancillary Studies of Thyroid FNAs: Conclusions

- Current state-of-the-art
  - Characterization of a known malignancy
    - IHC
- Future: Biomarkers to increase specificity for malignancy
  - Several candidates (protein, nucleic acids)
  - Need further development