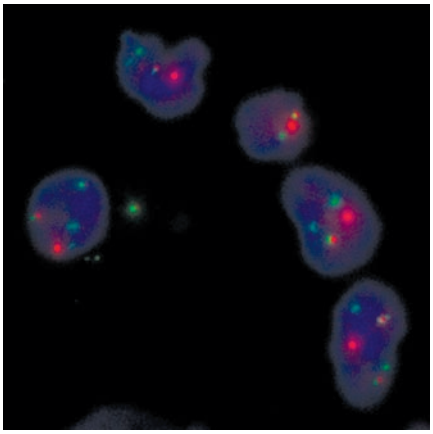
Phenomena™

THE NEWSLETTER OF PHENOPATH LABORATORIES

VOLUME 10 NO.4 1-888-92-PHENO WWW.PHENOPATH.COM NOVEMBER 2007

NEW FISH STUDIES OFFERED

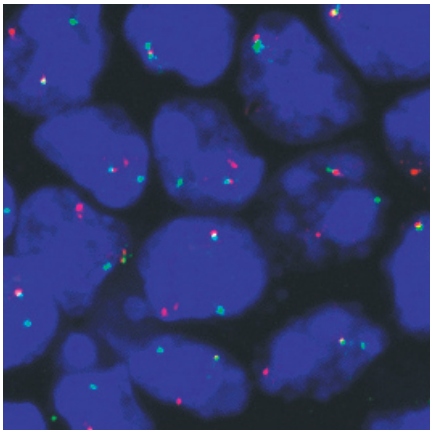
PhenoPath is pleased to announce the availability of two new FISH studies. These tests are available on formalin-fixed, paraffin-embedded (FFPE) tissues.



t(9;22) involving BCR/ABL in a bone marrow needle core biopsy

t(9;22) BCR/ABL FISH

Chronic myeloid leukemia (CML) is a myeloproliferative disorder representing 15-20% of adult leukemias. CML has been traditionally diagnosed by detection of the Philadelphia chromosome (Ph) which is the hallmark of this disease and is the result of a translocation between the BCR gene on chromosome 22 and the ABL gene on chromosome 9. FISH studies offer a highly specific and sensitive method for detecting this translocation in blood, bone marrow and FFPE tissues. The t(9;22) is also found in ~20% of adult precursor B cell acute lymphoblastic leukemias, and is associated with an unfavorable prognosis. In addition, the few patients with acute myeloid leukemia (AML) harboring a t(9;22) translocation involving BCR/ABL are known to have an unfavorable prognosis, and in a recent ECOG study, interphase FISH has been shown to be highly accurate in helping to stratify AML patients into cytogenetically defined risk categories (Vance GH et al., *Leuk Res 31:605-9, 2007*; Slovak ML et al., *Blood 96:4075–83, 2000*). In normal cell nuclei, this FISH assay yields two orange and two green signals, but in nuclei of cells harboring a reciprocal BCR/ABL translocation, FISH results in a single orange and single green signal and two orange/green fusion signals. In FFPE tissue, nuclei are often transected and thus, only a single fusion signal may be seen ***(see image)***.



t(15;17) in an acute promyelocytic leukemia

RARA Breakapart FISH

Acute promyelocytic leukemia (APL) is a distinct form of acute myeloid leukemia which accounts for 5 to 10% of cases and is characterized by a proliferation of myeloid precursors blocked at the promyelocyte stage of differentiation. The abnormal promyelocytes may be rare or numerous in the peripheral blood. Patients with APL are typically relatively young among AML patients and have a better prognosis, as long as the frequent complication of disseminated intravascular coagulation (DIC) is adequately managed. Therefore, proper subclassification of this leukemia is critical for optimal patient management (i.e., to alert the treating oncologist that there is a significant risk of DIC). Typically, these patients are treated with all-trans-retinoic acid (ATRA), a relatively well-tolerated therapeutic agent that promotes myeloid differentiation. Myeloid differentiation of these abnormal promyelocytes can be followed by standard chemotherapeutic agents with a reduced risk of DIC.

(continued on page 2)

(RARA Breakapart FISH continued from page 1)

FISH tests can be used to establish a definitive diagnosis of APL. There is a strong association of the t(15;17)(q22;q12) and its associated PML/RARA fusion gene with this subtype of AML. FISH studies are capable of detecting a t(15;17)(q22;q12) in almost all cases. The rapid nature of this FISH-based testing, along with its high specificity and sensitivity, are significant advantages in the clinical setting. Moreover, the use of RARA breakapart FISH probes permits the identification of variant RARA gene translocations that would be missed using standard t(11;18) dual color, dual fusion FISH probes. The three main variant translocations involving the RARA gene include: t(11;17)(q23;q21) involving the promyelocytic leukemia zinc finger gene (PLZF) on 11q23; t(5;17)(q23;q21) involving the nucleophosmin (NPM) on 5q23; and t(11;17)(q23;q21) involving the nuclear matrix-associated gene (NUMA) on 11q13. Acute promyelocytic leukemias involving the variant t(11;17)(q23;q21) have been reported to be resistant to ATRA whereas those with variant t(5;17)(q23;q21) appear to respond to ATRA. In normal cell nuclei, this breakapart probe set yields two paired orange/green signals, but in the nuclei of cells harboring a translocation involving the RARA gene, one paired (normal allele) and two unpaired signals are observed ***(see image on page one)***.

Recent Publication By PhenoPath Laboratories' Pathologists

Farinola MA, Gown AM, Judson K, Ronnett BM, Barry TS, Movahedi-Lankarani S, Vang R. Estrogen Receptor [alpha] and Progesterone Receptor Expression in Ovarian Adult Granulosa Cell Tumors and Sertoli-Leydig Cell Tumors

Intl J Gynecol Pathol 26:375-382, 2007

International Journal of Gynecological Pathology
26:375–382, Lippincott Williams & Wilkins, Baltimore
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Original Article

Estrogen Receptor α and Progesterone Receptor Expression in Ovarian Adult Granulosa Cell Tumors and Sertoli-Leydig Cell Tumors

Maryam A. Farinola, M.D., Allen M. Gown, M.D., Kara Judson, M.D., Brigitte M. Ronnett, M.D., Todd S. Barry, M.D., Ph.D., Saeid Movahedi-Lankarani, M.D., and Russell Vang, M.D.

Summary: The biologic role that estrogen receptor (ER) and progesterone receptor (PR) play in ovarian sex cord–stromal tumors is poorly understood. Furthermore, immunohistochemical data on these hormone receptors in this group of neoplasms are limited and conflicting, with many reports suggesting that expression of ERs and/or PR is either infrequent or present at low levels in granulosa and Sertoli cell tumors. Immunohistochemical staining for ERs and PR was performed in 69 ovarian sex cord–stromal tumors (41 adult granulosa cell tumors and 28 Sertoli-Leydig cell tumors). Extent of expression was scored based on the percentage of positive cells: 0, 5% or less; 1+, 6% to 25%; 2+, 26% to 50%; 3+, 51% to 75%; and 4+, 76% to 100%. Estrogen receptor α and PR were frequently expressed in adult granulosa cell tumors (66% and 98%, respectively) and Sertoli-Leydig cell tumors (79% and 86%, respectively). Diffuse (3+ or 4+) expression of PR was more common in adult granulosa cell tumors (68% vs. 36%; $P = 0.013$), whereas diffuse (3+ or 4+) expression of ERα was more frequent in Sertoli-Leydig cell tumors (50% vs. 20%; $P = 0.010$). In cases positive for both markers, adult granulosa cell tumors exhibited a focal (1+ or 2+) ERα/diffuse (3+ or 4+) PR constitutive profile more commonly than Sertoli-Leydig cell tumors (52% vs. 18%; $P = 0.02$), whereas Sertoli-Leydig cell tumors displayed a diffuse (3+ or 4+) ERα/focal (1+ or 2+) PR constitutive profile more frequently than adult granulosa cell tumors (50% vs. 0%; $P = 0.0007$). We conclude that expression of hormone receptors (based only on frequency of immunostaining) does not allow for distinction from other tumors in the differential diagnosis that are known to be frequently positive for ERs and PR such as endometrioid neoplasms. Most adult granulosa cell tumors and Sertoli-Leydig cell tumors share overlapping patterns of expression of ERs and PR with each other, but a subset of cases in each tumor category exhibits unique ERα/PR immunoprofiles (eg, focal ERα/diffuse PR in adult granulosa cell tumors and diffuse ERα/focal PR in Sertoli-Leydig cell tumors). These patterns of expression of ERα and PR may aid our understanding of the biologic differences between granulosa and Sertoli cell tumors. **Key Words:** Estrogen receptor—Progesterone receptor—Adult granulosa cell tumor—Sertoli-Leydig cell tumor—Immunohistochemistry.

From the Departments of Pathology (M.A.F., K.J., B.M.R., T.M.-L., R.V.) and Gynecology and Obstetrics (A.M.G.), The Johns Hopkins University School of Medicine, Baltimore, Maryland, and PhenoPath Laboratories and IMPRIS (A.M.G., T.S.B.), Seattle, Washington.

Address correspondence and reprint requests to Russell Vang, MD, Department of Pathology, Division of Gynecologic Pathology, The Johns Hopkins Hospital, Rm 2242, Weinberg Bldg, 401 North Broadway, Baltimore, MD 21231. E-mail: rvang1@jhmi.edu.

Estrogen receptor α (ERα) and progesterone receptor (PR) are commonly expressed in surface epithelial-stromal tumors of the ovary, mainly those of serous and endometrioid types; however, limited and conflicting information exists on expression of these hormone receptors in ovarian sex cord–stromal tumors, particularly adult granulosa cell tumors and Sertoli-Leydig cell

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DOI: 10.1097/pgp.0b013e31805ab999

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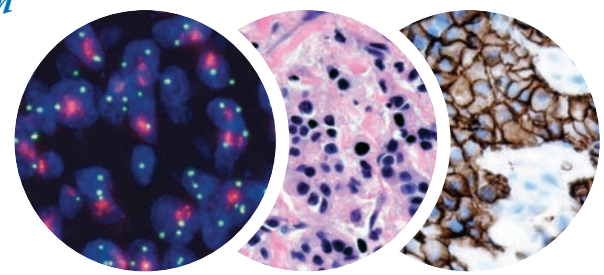
In this study, the product of the collaboration between the pathologists of PhenoPath Laboratories and the Department of Pathology at the Johns Hopkins University School of Medicine in Baltimore, MD, immunostaining for ER and PR was performed on 41 adult granulosa cell tumors and 28 Sertoli-Leydig cell tumors. ER and PR were shown to be expressed in adult granulosa cell tumors (66% and 98%, respectively) and Sertoli-Leydig cell tumors (79% and 86%, respectively). One finding was that the expression of hormone receptors (based only on frequency of immunostaining) does not allow for distinction from other tumors in the differential diagnosis that are known to be frequently positive for ER and PR (e.g., endometrioid neoplasms). In the sex cord stromal tumors examined, however, a subset of cases in each tumor category exhibited unique ER/PR immunoprofiles (e.g., focal ER/diffuse PR in adult granulosa cell tumors and diffuse ER/focal PR in Sertoli-Leydig cell tumors). These patterns of expression of ER and PR may aid our understanding of the biologic differences between granulosa and Sertoli-Leydig cell tumors.

HER2 IMMUNOHISTOCHEMISTRY VALIDATION

Update and Correction

In the previous issue of Phenomena, we cited the published ASCO-CAP guidelines that indicated that annual revalidation of HER2 IHC testing was required. Dr. Gown, who is a member of the CAP IHC Committee, has received clarification that the reference in the ASCO-CAP guidelines to annual revalidation actually refers to the annual proficiency testing that is required of all laboratories involved in HER2 IHC testing. The actual test validation, i.e., the comparison of IHC and FISH HER2 results on 25-100 cases, needs to be performed only once, unless modifications in methodology are made, which would then require revalidation.



HER2 FISH *H&E* *HER2 IHC*

PhenoPeople Profile

Akemi Allison-Tacha

PhenoPath Laboratories is pleased to welcome our new Client Services Manager, Akemi Allison-Tacha, B.S., HT (ASCP) HTL. She has worked closely with the pathology community for decades in clinical and research laboratories throughout the US and has gained a reputation for her love and passion for the histotechnology profession. Her goal at PhenoPath is to make a positive impact on patient care through her efforts in client services. With Akemi's unique understanding of histology, immunohistochemistry (IHC), and customer service, she plans to work with the dedicated team at PhenoPath in optimizing patient care.

PhenoPath Laboratories and our clients will greatly benefit from Akemi's expertise, as she manages the accessioning and transcription staff, monitors QA and QC functions, acts as liaison between PhenoPath's staff, pathologists and clients, along with a host of other functions necessary to provide outstanding service to PhenoPath's clients and their patients.

Akemi comes to PhenoPath from Phoenix Lab Consulting & Staffing Services in Danville, CA, where she served as President/Director. Prior to Phoenix Lab Consulting, Akemi served as Director of Histology at Biocare Medical in Concord. While at Biocare, she developed the histology product line and oversaw manufacturing, QC & QA, technical support, customer support, shipping and receiving, and marketing.

Akemi earned her BS degree from Tokyo University, Japan, and subsequently completed the Histology Residency Program at Binghamton General Hospital, Binghamton, NY. Akemi's many interests include a passion for histology, art, food and wine. She was on the foundation level of the Portland Saturday Market in Portland, Oregon, and has also owned and operated her own catering business. As an avid adventurer, Akemi has lived in many countries throughout the world.

PhenoPath Laboratories is pleased to welcome Akemi to the PhenoFamily.

VISIT US AT THE FOLLOWING MEETINGS:

For up-to-date information, visit our website www.phenopath.com.

Nassau County Society of Pathologists Meeting:
Diagnostic Immunohistochemistry for the Practicing Surgical Pathologist
October 20, 2007, North Shore University Hospital, Manhassett, NY
Allen M. Gown, MD was a featured speaker and presented three talks entitled "IHC Evaluation of Large Cell Undifferentiated Malignant Neoplasms," "IHC Evaluation of Small Blue Round Cell Tumors," and "Carcinomas of Unknown Primary." http://www.naspath.org/

Arizona Society of Pathologists Meeting
October 27-28, 2007, Hilton Scottsdale Resort, Scottsdale, AZ
Todd S. Barry, MD, PhD was a featured speaker and presented three talks entitled "Update and Troubleshooting in Immunohistochemistry," "Challenging Cases in Diagnostic Immunohistochemistry," and "Use of Interphase FISH in Diagnostic Pathology" on October 28, 2007.

International Symposium on Cancer Biology
November 14-16, 2007, National Institute of Immunology, New Delhi, India
Allen M. Gown, MD is a featured speaker and will present a talk entitled "Immunohistochemistry: A Window Onto the Molecular Biology of Tumors" on November 15, 2007.

California Society of Pathologists 60th Annual Convention: *California Seminars in Pathology*
December 5-8, 2007, The Palace Hotel, San Francisco, CA
PhenoPath will be represented at an exhibit booth. http://www.calpath.org/

San Antonio Breast Cancer Symposium
December 13-16, 2007, Henry B. Gonzalez Convention Center, San Antonio, TX
Abstract #5032 entitled "Quality control procedures are essential in accurate HER2 FISH testing using automated morphometric image analysis" by Todd S. Barry, MD, PhD, et al. is scheduled to be presented at Poster Session 5 & Reception on Saturday, December 15, 2007 from 5:00-7:00 PM (Exhibit Hall B). In addition, PhenoPath will be represented at an exhibit booth. http://sabcs.org/

Visiting Professor Program:
Department of Pathology at Montefiore Medical Center, Albert Einstein College of Medicine
January 10, 2008, Montefiore Medical Center, Bronx, NY
Allen M. Gown, MD is a featured speaker and will present a talk entitled "Current Issues in ER & HER2 Testing in Breast Cancer" on January 10, 2008. http://www.aecom.yu.edu/

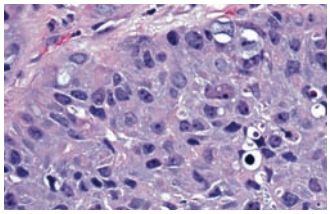
2nd Int'l Course on Applied IHC & Molecular Morphology
January 28-February 1, 2008, Fess Parker's Double Tree Resort, Santa Barbara, CA
Todd S. Barry, MD, PhD and Allen M. Gown, MD are featured speakers. Dr. Barry will present two talks entitled "Overview of FISH Technique," and "FISH Applications in Lymphomas/Leukemias." Dr. Gown will present a talk entitled "Molecular Applications in IHC (Genomic IHC)," and will participate in the "Expert Panel Discussion, Part II: Community Thought Leaders." http://www.appliedimmuno.org/

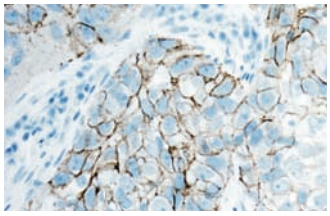
The 14th Annual Practical Pathology at Whistler
January 29-February 1, 2008, Fairmont Chateau Whistler Resort, Whistler, BC, Canada
Allen M. Gown, MD is a featured speaker and will present a talk entitled "Diagnostic Immunohistochemistry - What Can Go Wrong and How to Prevent It" on January 30, 2008. http://www.pathology.ubc.ca/

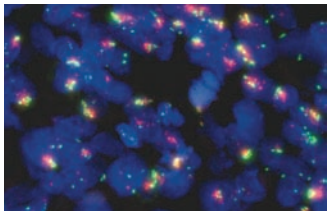
PhenoPath Laboratories' HER2 Testing Quality Assurance Program

In January 1999, with the introduction of routine HER2 testing of breast carcinomas, PhenoPath Laboratories commenced a quality assurance program to maximize the accuracy of testing by immunohistochemistry (IHC) and by fluorescence in situ hybridization (FISH). For all cases submitted to our laboratory for HER2 testing by FISH, an accompanying IHC test is performed in parallel. In addition, for all cases referred for IHC testing which demonstrate a 2+ (equivocal) score, a HER2 test by FISH is performed.
Quarterly reviews of the concordance data between IHC and FISH are performed by a pathologist. All cases in which there is a discrepancy between IHC and FISH, that is IHC (0, 1+) and FISH amplified or IHC 3+ and FISH non-amplified, are reviewed. The IHC slides and the digital images are reviewed by all the pathologists at our daily Pathology Case Conference. In addition, an audit of a subset of cases submitted for HER2 testing is also performed. The primary data and clinical records from approximately 10% of cases tested by both IHC and FISH and 5% of cases tested only by IHC are reviewed by a pathologist.

The initial results of this concordance data (Yaziji H et al, *JAMA 291;1972- 1977, 2004*) have recently been updated. An analysis of 6604 cases, from 2003 to 2006, showed a high rate of concordance between IHC and FISH. 1904/1919 (99.2%) of those showing IHC results of 0 or 1+ proved to be non-amplified by FISH, and 529/562 (94.7%) of those cases showing IHC results of 3+ proved to be amplified. These data were presented at the March 2007 USCAP meeting in San Diego and have been submitted for publication.
Recently published guidelines from ASCO and CAP mandate that laboratories performing HER2 tests show high concordance rates between IHC and FISH (95%.) At PhenoPath, we have achieved this extremely high rate of concordance, at least in part due to the institution of a comprehensive quality assurance program, ongoing for years, which permits continuous visual feedback of IHC results with FISH-determined HER2 gene status.


H&E


HER2 IHC


HER2 FISH

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Expertise, Innovation and Excellence

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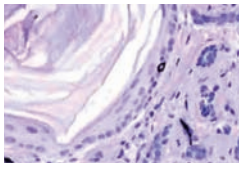
Featuring Dr. Mark R. Wick

At Our Fall Quarterly Conference

UNIVERSITY of VIRGINIA



Dr. Mark R. Wick, of the University of Virginia at Charlottesville, Virginia, will present "Diagnostic Immunohistochemistry in Dermatopathology: An Update" at the Quarterly Pathology/Immunohistochemistry Conference on *Thursday, November 29, 2007*. The format of the conference is a social hour commencing at 6:30 p.m. followed by Dr. Wick's lecture at 7:30 p.m. A light catered dinner will be served during the social hour.

Dr. Wick is currently a Professor of Pathology in the Divisions of Surgical Pathology and Cytopathology, and Autopsy Pathology, Associate Director of Surgical Pathology, and Director of Pathology Residency Training at the University of Virginia at Charlottesville. He received his M.D. from the University of Wisconsin, and completed his anatomic and clinical pathology residency training at the Mayo Clinic. Dr. Wick is a physician who has continued to practice as a general anatomic pathologist with particular interest in immunohistochemistry, dermatopathology, thoracic pathology and soft tissue pathology.

Dr. Wick's research focuses on protein chemistry and immunohistology of human neoplasms, and clinical outcomes analysis.

The Fall Quarterly Conference will be co-sponsored by Dako.