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PATHOLOGYBEAT

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Shining a Fluorescent Light: 10-color Flow Cytometry and Cluster Analysis
Nitin Karandikar, MD, PhD, Chair and Department Executive Officer

Multidisciplinary Approach to Diagnosis, Risk Stratification, and Minimal Residual Disease Detection in Plasma Cell Neoplasms
Carol Holman, MD, PhD, Director of Leukemia Pathology, Holden Comprehensive Cancer Center

Cytogenetic Studies in Neoplastic Lymphoid Conditions
Ben Darbro, MD, PhD, Director, Shivanand R. Patil Cytogenetics and Molecular Laboratory

Prognostic Markers in B-cell Lymphomas
Sergei Syrbu, MD, PhD, Medical Director, Immunopathology Laboratory

Myeloid Neoplasms: Morphology and Beyond
Nancy Rosenthal, MD, Director of Hematopathology

Next Generation Sequencing for High Yield AML and MDS Analysis
Aaron Bossler, MD, PhD, Director, Molecular Pathology Laboratory

Presentation summaries on page 2

Special Event!
FRIDAY, MAY 1
KINNICK STADIUM PRESS BOX
Case Presentations
Wine, hors d’oeuvres, socializing and reminiscing

Munir Tanas, MD joins faculty from Cleveland Clinic
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NEW FRONTIERS
Conference Presentation Summaries

Shining a Fluorescent Light: 10-color Flow Cytometry and Cluster Analysis
Nitin Karandikar, MD, PhD, Chair and Department Executive Officer
This talk will cover the utility of flow cytometry in the diagnosis and management of hematolymphoid neoplasia. The advantages of the cluster analysis approach with 10-color flow cytometry will be demonstrated.

Multidisciplinary Approach to Diagnosis, Risk Stratification, and Minimal Residual Disease Detection in Plasma Cell Neoplasms
Carol Holman, MD, PhD, Director of Leukemia Pathology Holden Comprehensive Cancer Center
Plasma cell neoplasms span a wide spectrum of clinical presentations and outcomes. Therefore, correct classification is essential in order for the proper treatment to be started and the appropriate prognostic information to be conveyed to the patient. This session will review the current classification of plasma cell neoplasms, and describe the role of cytogenetic, FISH, and flow cytometric testing in the initial evaluation of these patients. The role of flow cytometry and CD138-enriched FISH to evaluate minimal residual disease following treatment will also be discussed. Case-based examples will be used throughout the presentation to illustrate key points.

Cytogenetic Studies in Neoplastic Lymphoid Conditions
Ben Darbro, MD, PhD, Director, Shivanand R. Patil
Cytogenetics and Molecular Laboratory
Diagnosis, prognostication, and treatment decisions for neoplastic lymphoid conditions rely heavily on cytogenetics testing. Conventional cytogenetics (karyotype), fluorescence in situ hybridization (FISH), and now cytogenomic/chromosomal microarrays (ICMA) can all be components of clinically appropriate cytogenetic studies on these diverse neoplasms. In this session, Dr. Darbro will be presenting the current state of the art of cytogenetic-testing for acute and chronic lymphoid leukemias as well as non-Hodgkin lymphomas.

Prognostic Markers in B-cell Lymphomas
Sergei Syrba, MD, PhD, Medical Director, Immunopathology Laboratory
Updates on prognostic markers in B-cell lymphoma (DLBCL, MCL and FL), which includes:
1. Clinical markers – IPI, MIPI and FLIP scores
2. Tumor intrinsic markers – Cell of origin (GBC vs ABC type), FISH (cMYC, Bcl-2, and Bcl-6) and IHC for the expression/significance of Bcl-2, cMYC, Bcl-6, CD5, MIB1, p53, etc.
3. Tumor microenvironment – Tumor associated histocyte-macrophage cells (M1 vs M2 type)
4. Extra-tumoral markers – serum free immunoglobulin light chains, serum cytokines/chemokines (IL1RA, IL2R, IL8, MIP1-α), and CXCL9, and absolute lymphocyte/monocyte count.

Myeloid Neoplasms: Morphology and Beyond
Nancy Rosenthal, MD, Director of Hematopathology
The diagnosis of acute myeloid leukemia can be difficult and the parameters by which we subclassify these leukemias continues to evolve. In this session we will review clinical, morphologic, immunophenotypic and cytogenetic abnormalities that allow us to classify AML. We will also discuss new cytogenetic abnormalities that may be leukemia defining in the future. The clinical importance of morphologic versus cytogenetic abnormalities in AML with myelodysplasia related changes and the difficult diagnosis of acute erythroid leukemia will be presented. Finally, reactive mimics that may lead to the misdiagnosis of AML will be shown.

Next Generation Sequencing for High Yield AML and MDS Analysis
Aaron Bossler, MD, PhD, Director, Molecular Pathology Laboratory
This presentation will review the clinical utility and testing options for mutation profiling including common standard of care genetic changes and up and coming genetic biomarkers. Testing options available from the University of Iowa Molecular Pathology Laboratory will be discussed.

“Diagnosis of bone and soft tissue tumors is supported by a comprehensive immunohistochemical panel including STAT6 (solitary fibrous tumor/hemangiopericytoma), INI1 (renal rhabdoid tumor/malignant extrarenal rhabdoid tumor, epithelioid sarcoma, other epithelioid sarcomas), TLE1 (synovial sarcoma), MUC4 (low grade fibromyxoid sarcoma), NKI-C3 (cellular neurothekeoma), MDM2/CDK4 (well differentiated/dedifferentiated liposarcoma, and sclerosing epithelioid fibrosarcoma), TLE1 (synovial sarcoma), MUC4 (low grade fibromyxoid sarcoma, NKI-C3 (cellular neurothekeoma), MDM2/CDK4 (well differentiated/dedifferentiated liposarcoma, and sclerosing epithelioid fibrosarcoma).”

Figure 1. Osteoblastoma. Histological evaluation reveals a bone-forming lesion characterized by a proliferation of plump osteoblasts. Osteoblasts can be enlarged, imparting a hypercellular appearance to the lesion and mimicking a more aggressive neoplasm. However, cytological atypia is absent and correlation with radiology demonstrated a non-aggressive appearance to the lesion, supporting the diagnosis of osteoblastoma.

Figure 2. Low grade fibromyxoid sarcoma/expressing epithelioid fibrosarcoma. This hybrid sarcoma contains histological features of both low grade fibromyxoid sarcomas (panel A) and sclerosing epithelioid fibrosarcoma (panel B). The diagnosis is supported by strong and diffuse immunoreactivity for MUC4 (panel C), including the sclerosing epithelioid fibrosarcoma component (SIF). A subset of SIF, in particular those associated with low-grade fibromyxoid sarcoma (LGFS), histology have been shown to harbor the same t(6;14) translocation seen in LGFSMs, suggesting that these patterns represent the histological spectrum of the same entity.
Amyloidosis is a group of disorders with an inherent defect in protein folding and extracellular deposition of low molecular weight fibrils. These are composed of soluble precursor proteins that have undergone conformational change. There is progressive organ dysfunction due to normal tissue replacement by amyloid.

Historic reference to amyloid can be found in the writings of Rudolf Virchow in 1853, who referred to tissue deposits of starch like material that stained in a similar manner to plant cellulose when exposed to iodine. He also described their amorphous and hyaline appearance on light microscopy. Subsequently with the use of polarized microscopy these deposits exhibited apple green birefringence with Congo red dye. Clinical manifestations depend upon the type, location, and the amount of deposition of these amyloid fibrils. However, initial symptoms may be non-specific and the diagnosis may be missed. Although the disease incidence is estimated at 8 cases/ million people per year in the United States, it may be underdiagnosed. Progress in the diagnosis and treatment of amyloidosis has led to efficacious clinical response and long-term survival can be achieved.

Amyloid deposition in a particular organ depends not only on the type of amyloid, but also on the extracellular matrix. The amyloid fibrils are characterized as having an antiparallel beta-pleated sheet configuration. The amyloid precursor protein undergoes conformational changes due to acidification or other chemical modifications, point mutations, deletions, premature stop codons or proteolytic cleavage. This results in protein misfolding and makes them fibrillogenic. The influence of the surrounding matrix also contributes to amyloid deposition, particularly in the setting of glomerulonephritis or chronic inflammatory conditions. Protein misfolding may also occur in association with aberrant chaperone proteins and increased production of amyloidogenic precursors. Interaction of the aberrant proteins with extracellular matrix components including serum amyloid P-component (SAP), proteoglycans, and glycosaminoglycans influence the specific organ/tissue localization of amyloid deposits.

At least 30 different human and 10 different animal protein precursors of amyloid fibrils are now known. The nomenclature for amyloidogenic precursors. Interaction of the aberrant proteins with extracellular matrix components including serum amyloid P-component (SAP), proteoglycans, and glycosaminoglycans influence the specific organ/tissue localization of amyloid deposits.

Identification: A renal biopsy is performed to establish the presence and type of renal amyloid, which is important for prognosis and treatment. Various techniques are available to identify the type of amyloid deposits, including direct immunofluorescence on frozen tissue, immunohistochemistry on paraffin-embedded tissue via the commercially available immunoperoxidase or alkaline phosphatase detection kits, and laser microdissection/mass spectrometry (LMD/MS).

Although rarely performed, gross examination of kidneys involved by amyloid reveals enlarged kidneys with a pale, “waxy” cut surface. All renal compartments may be involved including glomeruli, tubules, interstitium and renal medulla where they may be seen around the vasa recta, loops of Henle, and collecting ducts.

On light microscopy amyloid appears as eosinophilic amorphous material that progressively replaces the mesangium on hematoxylin and eosin stain (Figure 1A). Examination of the biopsy may demonstrate periodic acid Schiff (PAS) stain negative (Figure 1C) and Jones silver stain (JMS) negative (Figure 1D) areas where normal mesangial matrix has been replaced by amyloid. The nodular glomerular mesangial expansion in case of amyloid must be carefully differentiated from diabetic nephropathy (silver and PAS positive) and other forms of nodular glomerulosclerosis. Similar deposits may be noted in arterioles (Figure 1B) and sometimes resemble hyalinosis. However, they have the characteristic staining properties with PAS, JMS and Congo red which can help distinguish the two.

Other special stains used include Thioflavin (T or S) fluorescent stain, which are highly sensitive, but relatively less specific as they may bind to other smaller oligomers, proteins with a higher beta sheet content etc. The gold standard for the diagnosis of amyloidosis is the Congo red stain. Amyloid deposits when stained with Congo red appear “salmon pink” (Figure 2A) and exhibit “apple-green” birefringence (Figure 2B) when viewed under polarized light. The Congo red stained sections when viewed by ultraviolet light microscope with tetramethylrhodamine isothiocyanate (TRITC) filter will make the deposits stand out in bright red color.

Electron microscopy is used to identify amyloid fibrils. Glomerular amyloid spicules can result from parallel alignment of amyloid fibrils in the sub-epithelial zone perpendicular to the glomerular basement membrane (Figure 2C). The fibrils are described as rigid and non-branching, with an average diameter of 7 to 10nm (Figure 2D).

Types of Amyloidosis:

Light Chain amyloidosis (AL amyloidosis) – The disease affects mainly individuals with an average age of 65 years (range 23 to 91 years). Clinical manifestations include fatigue and weight loss. Patients usually present with nephrotic range proteinuria, edema, hepatosplenomegaly, cardiac failure and occasionally carpal tunnel syndrome. Renal involvement presenting with proteinuria is seen in 70% of patients. Cardiac involvement is seen in up to 60% of the cases and a subset of patients may also present with cardiac failure. AL amyloidosis usually occurs in association with plasma cell dyscrasias. Rarely, it may be associated with Waldenström
macroglobulinemia or non-Hodgkin lymphoma. Only 5% of patients with AL amyloidosis will have overt multiple myeloma at the time of presentation. Rather, most present with monoclonal gammapathy of uncertain significance (MGUS). The deposition of a fibrinogen-derived amyloid in the vast majority of patients are composed of fragments of immunoglobulin light chains accounting for approximately 85% cases, followed by heavy chains and light chains (AHL) both or rarely fragments of heavy chains (AH) only.

Renal biopsy is of particular value in patients with monoclonal gammapathy of undetermined significance (MGUS) with accompanying renal dysfunction. These findings will usually trigger a cascade of further investigation often including imaging, serum and urine protein electrophoresis with immunofixation, bone marrow biopsy and serum free light chains (fLC) assay.

Appropriate clinical findings are a trigger for instituting appropriate patient management for underlying plasma cell clone disease and amyloidolytic disease.

AA amyloidosis – AA amyloidosis is the second most common type of renal amyloidosis, accounting for 5% to 7% of cases. This is derived from the acute-phase reactant serum amyloid A protein (SAA). Under normal circumstances SAA plays a role in inflammation and defense functions. Up regulation of SAA production (in the setting of inflammation), and protein misfolding together cause tissue deposits. In developing nations tuberculosis or other chronic infections cause AA amyloidosis, whereas in developed nations autoimmune diseases such as rheumatoid arthritis, ankylosing spondylitis, chronic juvenile arthritis, inflammatory bowel disease and familial Mediterranean fever (FMF) are thought to be responsible. Interestingly, hereditary auto-inflammatory diseases and periodic fever syndromes, including FMF carry an increased risk for the development of AA amyloidosis. In the setting of chronic infection or autoimmune disorders, proteinuria leading to nephrotic syndrome and renal insufficiency are suggestive of AA amyloidosis.

The renal deposits are similar to those described for the AL amyloid, and in the case of FMF involve small caliber vessels throughout the body. In the kidney they extensively deposit in the glomeruli, around the tubules and rarely may involve interstitium. Patients may present with GI symptoms including malabsorption, intestinal pseudo-obstruction, diarrhea, or bleeding. They may also develop hepatitis, malignancy, gastrointestinal and adrenal insufficiency. However, cardiac, skin or soft tissue involvement is relatively uncommon.

It is important to remember that in patients with rheumatoid arthritis there may often be amyloid AA deposits with co-existing immune-complex disease. Hence a thorough investigation is warranted. Diagnosis depends on finding both the amyloid and organ biopsy involvement and histological confirmation of amyloid deposits.

In contrast to AL amyloid where the emphasis is control of malignant cell clone, treatment options in AA amyloid address control/management of underlying predisposing disease. The objective is to decrease the acute phase reactant levels, including circulating serum SAA levels. Immunomodulatory drugs are being used in controlling the progression of amyloidosis-associated renal symptoms including proteinuria and improving long-term survival. Efficient anti-inflammatory therapy can delay or halt the development of AA amyloidosis and preserve organ function in patients with rheumatoid arthritis, chronic infection etc.

The category of non immunoglobulin associated non-AA and apparently sporadic amyloidosis types include dialysis related amyloidosis, A-Lect 2 and ApoAIV associated amyloidosis.

Dialysis-related amyloidosis – In patients with renal failure who undergo chronic hemodialysis treatment, β2-microglobulin (β2m) can form amyloid in osteocartilaginous structures. It helps in maintaining stability of the MHCII molecules. The majority of this circulating β2m is filtered through the glomeruli into the tubules and reabsorbed by the proximal tubular cells. During hemodialysis the membrane molecular weight cut-offs are below or near the molecular weight of β2m, hence it does not get efficiently eliminated and accumulates within the patient.

Patients with end stage kidney disease have uremia and require dialysis. The concentration of both heparin and urea is increased in such patients, and these two substances are known to accelerate fibrillogenesis. Patients complain of shoulder pain due to arthritis of the scapulohumeral joint and amyloid deposition in the rotator cuff. Physiological/ reactive rise in β2m levels occurs in conditions of increased cell turnover such as viral infections and hematopoietic malignancies, but is not associated with β2m amyloidosis.

A-Lect 2 amyloidosis – This is derived from leucocyte chemoattractant factor 2, and is a recently identified form of amyloidosis, with a predilection for kidney disease. This is seen in individuals of Mexican heritage or those from the northern Indian subcontinent of Punjab. These cases are associated with an autoinflammatory syndrome related to biologic or non-biologic arthritis which involve liver, spleen and colon. Treatment strategies for this condition are not yet well established.

Apolipoprotein AIV – It is derived from apolipoprotein APOAIV, a glycoprotein that is important for lipid metabolism. There is no mutation evident in the APOAIV gene. Of the cases reported in literature the renal involvement was secondary to myeloma with almost no involvement of glomeruli or vessels. Thus far, there is no evidence of family history or mutation in the APOAIV gene.

Heritable amyloidoses – Hereditary (familial) forms are the predominant form of renal amyloidosis in the family. A-A amyloidosis is the second most common form of amyloidosis, which often but not always be suggested by the history and physical examination. These patients can be associated with familial diseases such as familial Mediterranean fever (FMF), familial hypercholesterolemia and familial hypertriglyceridemia and hypertension can further accelerate the fibrinogen variant in vascular walls and in atherosomatous plaques. Nephrotic syndrome with hyperlipidemia and hypertension can further accelerate the plaque formation and in susceptible patients give rise to AFib amyloid.

Usually these patients have no previous family history of kidney disease. They develop different phenotypes depending on the mutation. AFib is associated with a relatively slow progression of amyloid deposition in the kidney, compared to AL amyloid.

Age-related (senile) systemic amyloidosis – It is important to note that the wild type transthyretin has extensive β-pleated sheet structure and with age can undergo spontaneous aggregation and deposition in the myocardium. This is referred to as systemic senile amyloidosis (SSA). It occurs in older adults.

In summary amyloid nephropathy is common in AA and AL type amyloidosis, and relatively rare in ATTR, ALECT2, A-fibrinogen and A- apolipoproteins (AA, AI, and AIV) amyloidosis. After the introduction of mass spectrometry however, the rarer subtypes of amyloidosis, ALECT2 in particular, are being increasingly reported.

DIAGNOSIS – Purely clinical symptom based diagnosis of amyloidosis type is not possible. The presence of amyloidosis may often but not always be suggested by the history and physical examination. The laboratory findings must be interpreted with caution and with a full awareness of technique/instrument limitations and pitfalls.

In cases that are inconclusive or negative, evaluation by a reference laboratory, using more sophisticated methods such as DNA studies or mass spectrometry (MS) may be employed. MS identifies with precision the unique protein sub-structure present in hereditary and familial forms of amyloid. In addition, it can be performed on archival paraffin embedded tissue and obviates the need for fresh or frozen specimens.

The findings must be interpreted with caution and with a full awareness of technique/instrument limitations and pitfalls. Clearly accurate identification of the amyloid type is the key to appropriate patient management. More entities/mutations are likely to emerge as research into this field continues, and as targeted therapies are developed. The ultimate goal would be utilizing an appropriate combination of clinical skills, laboratory investigations and targeted therapy to reverse the disease course and ameliorate patient suffering.
Species-level identification of bacteria and yeast is one of the primary activities of the clinical microbiology laboratory. Biochemical techniques were developed during the first century of clinical microbiology that allowed presumptive identification of a few common microbes within minutes and more definitive identification within 8 hours to a day. This system has low resolution for closely-related species and often fails with inert or metabolically similar organisms such as non-glucose-fermenting Gram-negative rods and Gram-positive rods. Delay and uncertainty over identification are two common outcomes of failed or limited biochemistry that have historically delayed the initiation of effective, narrow-spectrum antibiotic treatment for pathogens and, for contaminants and commensal organisms, may result in unnecessary treatment and distraction from alternative diagnoses.

Another major activity of the laboratory, susceptibility testing of bacteria, is predicated on having at least a presumptive identification before testing is performed and reported, making rapid identification by means other than biochemistry an ever more attractive proposition.

The main historical alternative to biochemical identification, 16S (for bacteria) and 18S (for fungi) rDNA sequencing, is available to the UIHC Microbiology Laboratory and is expensive ($80 for in-house sequencing), low throughput, and takes at least two days but is almost always definitive. Because differences in ribosomal sequence can be used in this manner to identify bacteria and fungi and up to 50% of the dry weight of a growing bacterial cell consists of ribosomes, a proteomic technique that weights the major components of cells has the potential to identify virtually any bacterium or fungus without being dependent on biochemistry. Matrix-Assisted Laser Desorption Ionization / Time-of-Flight Mass Spectrometry (MALDI-TOF MS) is capable of doing this for organisms that have been recovered in culture in minutes instead of hours or days. A MALDI-TOF mass spectrum is generated as described in Figure 1 and contains the precise weights of each component of the cell that can be easily ionized within the mass range of 2-20kDa where most ribosomal components fall.

Because MALDI-TOF mass spectra are compared to a database of reference organisms’ spectra to make an identification, organisms must first be grown in pure culture before spectra are collected and an identification is made. If multiple organisms are present the extra mass peaks render no spectrum in the database a good match and the identification fails. Because blood culture bottles typically (90% of the time) contain a large number of bacteria of a single species, a commercial extraction system was implemented [4][5][6][7].

SepsiTyper testing was recently completed and validated at the University of Illinois Hospitals and Clinics (UIHC; directed by Dr. Bradley Ford) and the University of Iowa Hospitals and Clinics (UIHC; directed by Dr. Stacey Klutts) and the University of Iowa Hospitals and Clinics are the only hospitals in Iowa that have implemented MALDI-TOF mass spectrometry for routine microbial identifications. UIHC currently performs about 2,000 identifications per month by MALDI-TOF MS. Hiring of personnel to perform Sepsityper testing was recently completed and validation of the initial construction of a collaborative network of physicians and pharmacists to manage the data is a current work in progress.

The laboratories at the Iowa City Veterans’ Administration (VA; directed by Dr. Stacey Klutts) and the University of Iowa Hospitals and Clinics (UIHC; directed by Dr. Bradley Ford) are the only hospitals in Iowa that have implemented MALDI-TOF mass spectrometry for routine microbial identifications.

Figure 1: A laser light source is applied to B) a spot on a stainless steel target containing organisms overlaid with α-Cyano-4-hydroxycinnamic acid (HCCA) matrix which desorbs such that the larger components [C] accelerate more slowly than the smaller components [D] in a high voltage (arrow) across an evacuated tube. A detector [E] measures the time of flight between desorption and detection, which is used to calculate a mass spectrum.

MALDI-TOF in Clinical Microbiology

Bradley Ford, MD, PhD
Medical Director. Clinical Microbiology
Clinical Assistant Professor, Pathology
Email bradford@uiowa.edu

Reduction of antibiotic use through rapid M. tuberculosis identification


Impact of rapid microbial identification from positive blood cultures using matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry on patient management.


Integrating Rapid Pathogen Identification and Antimicrobial Stewardship Significantly Decreases Hospital Costs.


Comparison of the MALDI Biotyper System Using SepsiTyper Specimen Processing to Routine Microbiological Methods for Identification of Bacteria from Positive Blood Culture Bottles.

ERG: Background: The ETS-family transcription factor ERG (ETS-related gene) is constitutively expressed by endothelial cells and related neoplasms, and ERG gene fusions are identified in 40-50% of prostate cancers (TMPRSS2-ERG) and a smaller number of acute myeloid leukemias and Ewing sarcomas (10%).

ERG has emerged as the preferred marker of endothelial differentiation (Images 1A-D), compared to alternative markers including CD34, CD31, factor VIII-related antigen, and FLI1. Advantages include its high sensitivity and specificity and, as a transcription factor, ease of interpretation of staining. ERG has also been suggested as a secondary prostate cancer marker, either in the setting of small foci in a pleomorphic malignant neoplasm. A diagnosis of poorly differentiated non-small cell carcinoma is rendered based on a positive pan-keratin (Images 1A-D), compared to alternative markers including CD34, CD31, factor VIII-related antigen, and FLI1. Advantages include its high sensitivity and specificity and, as a transcription factor, ease of interpretation of staining. ERG has also been suggested as a secondary prostate cancer marker, either in the setting of small foci in a pleomorphic malignant neoplasm. A diagnosis of poorly differentiated non-small cell carcinoma is rendered based on a positive pan-keratin.

Miettinen and colleagues examined ERG expression in 21 dedifferentiated liposarcomas (DDLPS), and 10% of 10 deep fibrous histiocytomas and not in any of 140 other potential histologic mimics including cellular angiofibroma, desmoid fibromatosis, DFSP, gastrointestinal stromal tumor, low-grade fibromyxoid sarcoma, malignant peripheral nerve sheath tumor, monophasic synovial sarcoma, sarcomatoid mesothelioma, Schwannoma, soft tissue perineurioma, and spindle cell lipoma. STAT6 expression in a subset of DDLPSs has more recently been shown to be due to STAT6 amplification (the gene is at 12q13, nearby to 12q15, the latter consistently amplified in DDLPS).


SALL4: Background: The embryonic transcription factor SALL4 (sal-like protein 4) is a key regulator of pluripotency. While in the 10-week embryo SALL4 is expressed by germ cells, intestine, kidney, and some hepatocytes, in adult tissues SALL4 expression is limited to germ cells.

SALL4 has emerged as a highly sensitive marker of germ cell tumors (Images 3A-B), similar to placental alkaline phosphatase (PLAP). As SALL4 is a transcription factor, an advantage over PLAP is ease of interpretation. Additionally, SALL4 has superior sensitivity to PLAP in yolk sac tumor. It is variably expressed in chorionicarcoma and teratoma.

Miettinen and colleagues examined SALL4 expression in a set of 3,215 human tumors. As expected, SALL4 was expressed by all seminomas (n=85), embryonal carcinomas (n=30), and yolk sac tumors (n=9) and by most chorionicarcinomas (6%);


Stashek and colleagues detected ISL1 expression in 91% of 57 primary and 85% of 13 metastatic pancreatic NETs and in only 2% of 107 jejunoileal tumors. It was also expressed by 82% and 87% of duodenal and rectal tumors, respectively, although, of note, tumors from these sites rarely present as metastases of unknown origin. In the same study, PAX6 was expressed by 79% of 57 primary and 69% of 13 metastatic pancreatic NETs and by none of 107 jejunoileal tumors. It was also expressed by 62% and 56% of duodenal and rectal tumors.

Maxwell and colleagues subsequently found that an immunohistochemistry (IHC) classifier including the pancreatic NET markers ISL1, PAX6, PR, NES/P55, and PDX1 and the midgut NET markers CDX2 and PR/P can successfully assigned a pancreatic or midgut origin in 94% of 123 NETs. Of note, ISL1 and/or PAX6 positivity was detected in 90% of 31 pancreatic NETs, positioning these two markers in the first-tier, along with CDX2, of the IHC classifier.


**Islet 1 and PAX6:**

**Background:** The homeodomain-containing transcription factor Islet 1 (ISL1) is expressed in the islets of Langerhans, cells in the anterior and intermediate lobes of the pituitary, parafollicular cells in the thyroid, chromaffin cells in the adrenal medulla, and in subsets of neurons. An ISL1 knockout mouse demonstrates a complete absence of differentiated islet cells.

PAX6, 1 of 9 paired box genes, is a transcription factor critical in eye, brain, and islet of Langerhans development. A PAX6 knockout mouse lacks glucagon-producing α-cells.

Islet 1 and PAX6 have emerged as sensitive and specific markers of pancreatic (well-differentiated) neuroendocrine tumors (NET), especially in their distinction from midgut NETs (Images 5A-D).

**Image 5: ISL1 and PAX6 Staining in a Metastatic Neuroendocrine Tumor (NET) of Unknown Primary**

Image 4: S-100 vs. SOX10 Staining in a Clear Cell Sarcoma. (A) S-100, (B) SOX10 (each 40x). Transcription factors often demonstrate diffuse, strong staining in foci that only show weak, patchy staining with traditional differentiation markers.

Image 6: S-100 vs. SOX10 Staining in a Clear Cell Sarcoma. (A) S-100, (B) SOX10 (each 40x). Transcription factors often demonstrate diffuse, strong staining in foci that only show weak, patchy staining with traditional differentiation markers.

SOX10 has emerged as a highly sensitive marker of cranial neural crest and mesenchymal cells, melanocytes, and myoepithelial cells. SOX10 inactivating mutations cause Waardenburg syndrome, type IVc, characterized by deafness, hypopigmentation, and Hirschsprung disease. Activating mutations have been identified in some melanomas.

SOX10 has also been identified in a subset of examined SALL4-negative non-gem cell tumors. Expression was rare in 680 mesenchymal and neuroectodermal tumors with the notable exceptions of Wilms tumor (11/18; 61%) and rhabdoid tumor (3/3; 100%).

SOX10 was also expressed by 78 melanomas, 49% of 77 malignant peripheral nerve sheath tumors, 75% of 123 neuroendocrine tumors, 61% of 18 Wilms tumors, and 100% of 3 rhabdoid tumors. It is significantly more sensitive than other markers of melanocytic differentiation, including MiTF, melan-A, HMB-45, and tyrosinase.

S-100 also labels dendritic cells, fat, cartilage, and a subset of melanomas. Compared to S-100, SOX10 boasts both superior sensitivity (see below) and specificity, with strong staining in foci that only show weak, patchy staining with traditional immunohistochemistry (IHC) classifier including the pancreatic NET markers ISL1, PAX6, PR, NES/P55, and PDX1 and the midgut NET markers CDX2 and PR/P.

SOX10 is a highly sensitive and fairly specific marker of gem cell tumors. Expression is typically diffuse and strong, while expression in non-gem cell tumors is often focal (though occasionally diffuse, strong). SOX10-positivity in these latter tumors may reflect an embryonic stem cell phenotype.

**SOX10:**

**Background:** SOX10 (sex-determining region Y-related high mobility group box 10) is a transcription factor essential for neural crest development and phenotype maintenance. In normal tissues it is expressed by Schwann cells, melanocytes, and myoepithelial cells. SOX10 inactivating mutations cause Waardenburg syndrome, type IVc, characterized by deafness, hypopigmentation, and Hirschsprung disease. Activating mutations have been identified in some melanomas.


NEW Research Awards

Dr. Vladimir Badovinac received a Co-PI grant funding with Dr. John Hartry from the Department of Microbiology. The grant was awarded from the National Institutes of Health/National Institute of Allergy and Infectious Diseases (NIH/NIAID). The title of this project is Memory, CD8⁺ T-cell localization and protection from influenza. This funding total is $1,900,000 and is for the period of November 1, 2014 through October 31, 2019.

Dr. Nitin Karandikar received a notice of grant funding from the National Multiple Sclerosis Society. The title of this project is Role of CNS-specific autoreactive CD8⁺ T Cells in MS. The amount of this award is $712,800 and is for the period of April 1, 2013 through March 31, 2017.

Dr. Nitin Karandikar received grant funding from the National Institute of Health/National Institute of Allergy and Infectious Diseases (NIH/NIAID). The title of this project is CNS-specific regulatory CD8⁺ T cells in autoimmune demyelination. The total direct costs for this award are $1,125,000. The period for this project is May 1, 2011 through April 30, 2016.

Dr. Nitin Karandikar received grant funding from National Institute of Health/National Institute of Allergy and Infectious Diseases (NIH/NIAID). The title of this project is Cortical neurons direct the migration of autoreactive CD8⁺ T cells in the CNS. The total direct costs for this award are $2,500. The award is in the amount of $50,000.

Dr. Leslie Bruch received a Medical Student Interest Group (MSIG) award from the Inter society Council for Pathology Information, Inc. (ICPi). This 2014 MSIG award is a useful mechanism to encourage outstanding medical students to consider a career in pathology. The award is in the amount of $1,000.

Dr. Steven Moore received funding for a study in collaboration with Sarepta Therapeutics, Inc. The research funding is in the amount of $334,191.

Dr. Andrean Simons-Burnett received grant funding from the National Institutes of Health/National Institute of Dental & Craniofacial Research (NIH/NIDCR). The title of this project is Roles of inflammation in resistance to EGFR inhibitors in head and neck cancer. This funding total is $1,878,808 and is for the period of July 2, 2014 through April 30, 2019.

Dr. Munir Tanag received Sarcoma Pilot Funding from the University of Iowa, Melanoma and Sarcoma Program. The title of this project is Towards the clinical application of WWTR1 and the hippo pathway in breast cancer: a translational proposal. The award is in the amount of $30,000.

Dr. Weizhou Zhang received a V Scholar Grant Award from the V Foundation for Cancer Research. The title of this project is Metformin and Nlrp4 inflammasome in obesity-associated cancer progression. The award is in the amount of $200,000.

Dr. Weizhou Zhang received one of first small Thoracic MOG research grants at the University of Iowa. The title of this project is Metformin-associated protein 1 expression in lung adenocarcinoma. The award is in the amount of $15,000.

Dr. Kevin Legge received one of first small Thoracic MOG research grants at the University of Iowa. The title of this project is Chronic ethanol consumption and pulmonary immune suppression. This funding total is $396,376 and is for the period of September 5, 2013 through August 31, 2015.

Dr. Deoin Ma received research funding from the Carver College of Medicine, Holden Comprehensive Cancer Center at the University of Iowa. The title of this project is Molecular studies of leiomyosarcoma: identification of potential targets for personalized medicine. The award is in the amount of $10,000.

ACTIVE Research Awards

Dr. Kevin Legge received grant funding from the National Institutes of Health/National Institute of Alcohol Abuse and Alcoholism (NIH/NIAAA). The title of this project is Chronic ethanol consumption and pulmonary immune suppression. This funding total is $396,376 and is for the period of September 5, 2013 through August 31, 2015.

Dr. Marina Imanovic received research funding from the National Institutes of Health/National Institute of Alcohol Abuse and Alcoholism (NIH/NIAAA). The title of this project is Chronic ethanol consumption and pulmonary immune suppression. This funding total is $396,376 and is for the period of September 5, 2013 through August 31, 2015.


Research Publications continued


A simple and cost-effective method of DNA extraction from small formalin-fixed paraffin-embedded tissue for molecular oncologic testing. Snow AN, Stence AS, Pispersson JS, Bossler AD and Ma D. BMC Clinical Pathology 2014 Jul 7;14:30 PMID:25067909.


Amyloidosis And Kidney Disease: A Brief Review continued from page 7

Key References:

Pickens M. Amyloidosis—Where Are We Now and Where Are We Heading? Arch Pathol Lab Med. 2010;134:545–551.


The Department of Pathology celebrated recent faculty promotions at a faculty meeting on August 12, 2014. Congratulations to Vladimir Badovinac, PhD, who has been promoted to Associate Professor of Pathology and to Andrew Bellizzi, MD, who has been promoted to Clinical Associate Professor of Pathology, effective July 1, 2014.

The National Marrow Donor Program (NMDP) has recognized the DeGowin Blood Center with two achievement awards! The awards recognize our center for outstanding achievement in service and performance, as well as collecting more than 25 hematopoietic stem cell products for NMDP in 2013.

Thanks to our outstanding staff in the Blood Center and the Iowa Marrow Donor Program. Your hard work and dedication have made us a leader in the field.

Laila Dahmoush, MBChB, was appointed the University of Iowa Representative to the IAP (Iowa Association of Pathologists) board. Dr. Dahmoush replaces Robert A. Robinson, MD, PhD who has been the university’s IAP representative for five years. The Iowa Association of Pathologists is the leading statewide organization serving pathologists, patients and the public. Nationally, IAP is affiliated with the College of American Pathologists.

Dr. Prerna Rastogi received her medical training in India. She then moved to the United States where she pursued her doctoral studies at St. Louis University, St. Louis, MO in the lab of Dr. Jane McHowat, where she published several manuscripts, reviews and book chapters. She also received the American Heart Association pre-doctoral fellowship award. After receiving her PhD degree in 2008 she began her residency training in anatomic and clinical pathology at St. Louis University (2009-2013). Besides actively participating in medical and dental student teaching she served as the chief resident (2012-2013). Dr. Rastogi then joined the hematopathology fellowship at Moffitt Cancer Center in Tampa, FL in 2013, followed by specialized training in renal pathology at Nephropath in Little Rock, AR. She will participate on renal pathology and hematopathology services. She is married to Dr. Rahul Rastogi, a pain management physician, who will also be joining the UI Department of Anesthesia. They have 2 boys, Paarin (11 years) and Praneel (6 years). In her “me time” Dr. Rastogi likes to paint and drink tea. As a family they love to travel, her last visit being to Argentina.

Laila Dahmoush, MBChB, was appointed the University of Iowa Representative to the IAP (Iowa Association of Pathologists) board.

Dr. Prerna Rastogi was appointed the University of Iowa Representative to the IAP (Iowa Association of Pathologists) board.

The Department of Pathology Welcomes Dr. Prerna Rastogi
Monday, November 10, 2014

Dr. Prerna Rastogi received her medical training in India. She then moved to the United States where she pursued her doctoral studies at St. Louis University, St. Louis, MO in the lab of Dr. Jane McHowat, where she published several manuscripts, reviews and book chapters. She also received the American Heart Association pre-doctoral fellowship award. After receiving her PhD degree in 2008 she began her residency training in anatomic and clinical pathology at St. Louis University (2009-2013). Besides actively participating in medical and dental student teaching she served as the chief resident (2012-2013). Dr. Rastogi then joined the hematopathology fellowship at Moffitt Cancer Center in Tampa, FL in 2013, followed by specialized training in renal pathology at Nephropath in Little Rock, AR. She will participate on renal pathology and hematopathology services. She is married to Dr. Rahul Rastogi, a pain management physician, who will also be joining the UI Department of Anesthesia. They have 2 boys, Paarin (11 years) and Praneel (6 years). In her “me time” Dr. Rastogi likes to paint and drink tea. As a family they love to travel, her last visit being to Argentina.

Laila Dahmoush, MBChB, was appointed the University of Iowa Representative to the IAP (Iowa Association of Pathologists) board.

Tuesday, December 02, 2014

Laila Dahmoush, MBChB, was appointed the University of Iowa Representative to the IAP (Iowa Association of Pathologists) board. Dr. Dahmoush replaces Robert A. Robinson, MD, PhD who has been the university’s IAP representative for five years. The Iowa Association of Pathologists is the leading statewide organization serving pathologists, patients and the public. Nationally, IAP is affiliated with the College of American Pathologists.
For nearly 125 years, the University of Iowa Department of Pathology has been providing comprehensive diagnostic and treatment services to patients, as well as integrating clinical and scientific expertise with research. Each day, it’s evident that the UI Department of Pathology changes lives through education, patient care, and research.

What we do at the UI Department of Pathology wouldn’t be possible without alumni and friends like you. Your generous contributions—which allow us to maintain state-of-the-art facilities and keep pressing forward on numerous research endeavors—are now more important than ever. As state funding continues to decrease and National Institute of Health funding proves to be even more difficult to secure, your support allows us to continue our tradition of excellence, expand our opportunities for crucial research, and educate future generations of pathologists.

I look forward to my new role as Associate Director of Development for Major Gifts at the Roy J. and Lucille A. Carver College of Medicine. Together, we can collaborate with the UI Department of Pathology as it continues to excel in compassionate care, education, and research. It’s an honor to build upon the success of an exceptional program that is transforming the future of healthcare for generations to come.

To learn more about how your private support can change lives and support a department that is a recognized leader in pathology, please contact John Dwyer at john-dwyer@uiowa.edu or at (319) 467-3861.

A Letter from UI Foundation
Forever Building a Strong Future

John Dwyer
Associate Director of Development for Major Gifts for the Carver College of Medicine, University of Iowa Hospitals and Clinics, the University of Iowa Foundation
The University of Iowa Foundation
PO Box 4550
Iowa City, IA 52244-4550
319-467-3861

Each day, it’s evident that the UI Department of Pathology changes lives through education, patient care, and research.