Surgery Research Conference

Next Generation Sequencing Methodologies for Genomic Sciences and Precision Medicine

ACCME Accreditation Statement: The Medical College of Wisconsin is accredited by the Accreditation Council for Continuing Medical Education to provide continuing medical education for physicians. AMA Credit Designation Statement: The Medical College of Wisconsin designates this live activity for a maximum of 1.0 AMA PRA Category 1 Credit™. Physicians should claim only the credit commensurate with the extent of their participation in the activity. Hours of Participation for Allied Health Care Professionals: The Medical College of Wisconsin designates this activity for up to 1.0 hours of participation for continuing education for allied health professionals.
Research Highlights
Congratulations Tammy Kindel!

2019 recipient of the Society of American Gastrointestinal and Endoscopic Surgeons (SAGES) Young Researcher Award

*Abstracts due 1/16!

This award is designated for a young member of SAGES within 5 years of completion of fellowship training with significant clinical and/or basic science research, publication or presentation at national meetings, and dedication to an academic career.
Smart Cities-Smart Futures Round One Winning Teams captained by:

Michelle Botts
Student, CT

Andrew Resnick, MD, MBA
General

Ronald Woods, MD, PhD
Pediatric Congenital Cardiac
Congratulations to everyone at the 14th Annual Academic Surgical Congress!

February 5-7, 2019, Houston, Texas

2019: 41 accepted abstracts
2018: 25 accepted abstracts

Click HERE
MCW Cancer Center:
NCI Grant Support Request Form

Provide intensive support and resources to help with all aspects of pre-award development for our members submitting grants to the NCI. This includes support with editing, scientific review and administrative coordination. Please let us know how we can help you in your grant process!

For more information, contact Kitty Marquardt, research Program Manager
Come out & support MCW Pancreas Research

SUNDAY, JAN. 27 2019 // FREE TO SPECTATORS
HIGH/VOLTAGE
MOTORCYCLE ICE RACES
WILSON PARK LAGOON, MILWAUKEE // 16TH & HOWARD

January 27th
11:00-3:00pm
FREE
Wilson Park
Lagoon(16th & Howard)
Milwaukee
**Publications**

**Transplant Surgery**

**Impact of a Transplantation Critical Care Model on Short-Term Outcomes Following Liver Transplantation in High Acuity Patients:** *A Single-Center Experience.*  *Transplantation Proceedings*

(Zimmerman MA, Selim M, Kim J, Saieian K, Cinquegrani MP, Connolly L, Woehlck HJ, Lauer KK, Hong JC)

**Vascular Surgery**

**Endovascular Thrombus Removal for Acute Iliofemoral Deep Vein Thrombosis:** Analysis from a Stratified Multicenter Randomized Trial *Circulation*


**Pediatric Surgery**

**Corrigendum to "Red/near infrared light stimulates release of an endothelium dependent vasodilator and rescues vascular dysfunction in a diabetes model.*** *Free Radical Biology & Medicine*

(Keszler A, Lindemer B, Weihrauch D, Jones D, Hogg N, Lohr NL)

**Neural monitoring during H-type tracheoesophageal fistula division: A way to decrease recurrent laryngeal nerve injury?*** *Journal of Pediatric Surgery*

(Wright TN, Grant C, Hirshcl RB, Lal DR, Minneci PC, Fallat ME, Midwest Pediatric Surgery Consortium)

**Cardiothoracic Surgery**

**Porcine Small Intestinal Submucosa May Be a Suitable Material for Norwood Arch Reconstruction.*** *Annals of Thoracic Surgery*

(Jacobsen RM, Mitchell ME, Woods RK, Loomba RS, Tweddell JS)

**Surgical Oncology**

**A serum-induced transcriptome and serum cytokine signature obtained at diagnosis correlates with the development of early pancreatic ductal adenocarcinoma metastasis.*** *Cancer Epidemiology, Biomarkers & Prevention.*


**Colorectal Surgery**

**Race Is Associated With Burden of Anal Condyloma and Need for Operative Intervention.*** *Journal of Surgical Research*

(Foss HE, Blank JJ, Lundeen SJ, Peterson CY, Ludwig KA, Ridolfi TJ)
“The Word on Medicine: where Knowledge is changing life”

Saturday January 12 at 4:00 pm

The Word on Medicine will discuss the epidemic of adolescent obesity and what medical experts are doing about it. Panelists for this show include

Dr. Tammy Kindel  
Dr. Bethany Auble  
Dr. Bernadette Vitola  
Dr. Farhat Khan  
Dr. Elizabeth Fischer  
Denise Kilway (APN)
Next Month:

2017 We Care Award Update

Mitochondrial Telomerase as Regulator of Mitochondrial Damage and Secondary Messengers in Chemotherapy Induced Microvascular Dysfunction

February 13th, 2019
Conference Room M
5:00-6:00pm

Amanda Kong, MD, MS, FACS
Surgical Oncology

Andreas Beyer, PhD
Cardiology
NEXT GENERATION
SEQUENCING METHODOLOGIES
FOR GENOMIC SCIENCES
AND PRECISION MEDICINE

ANGELA J. MATHISON, PHD
ASSISTANT PROFESSOR, DEPARTMENT OF SURGERY | DIVISION OF RESEARCH
DIRECTOR OF RESEARCH AND DEVELOPMENT, GENOMIC SCIENCE AND PRECISION MEDICINE CENTER (GSPMC)

JANUARY 9, 2019
OUTLINE

• Genetic Alterations
  • Genome, Exome, Targeted panel
  • RNA seq – transcriptome

• Epigenetic Alterations
  • Other RNA species
  • DNA methylation
  • Histone modifications
GENETIC ALTERATIONS

Where do genetic alterations come from?

- Inheritance
- Replication
- DNA damaging agents

GENETIC ALTERATIONS

Defined: alteration of the genetic code (DNA) for a gene

- **silent:** has no effect on the protein sequence
- **missense:** results in an amino acid substitution
- **nonsense:** substitutes a stop codon for an amino acid
- **Insertion** or **deletion** results in a shift in the reading frame.
GENETIC ALTERATIONS

Defined: alteration of the genetic code (DNA) for a gene

### Genetic Alterations

How do we find these DNA alterations?

<table>
<thead>
<tr>
<th>Whole genome sequencing</th>
<th>Whole exome sequencing</th>
<th>Targeted sequencing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sequencing region: whole genome</td>
<td>Sequencing region: whole exome</td>
<td>Sequencing region: specific regions (could be customized)</td>
</tr>
<tr>
<td>Sequencing Depth: &gt;30X</td>
<td>Sequencing Depth: &gt;50X ~ 100X</td>
<td>Sequencing Depth: &gt;500X</td>
</tr>
<tr>
<td>Covers everything – can identify all kinds of variants including SNPs, INDELs and SV.</td>
<td>Identify all kinds of variants including SNPs, INDELs and SV in coding region.</td>
<td>Identify all kinds of variants including SNPs, INDELs in specific regions</td>
</tr>
<tr>
<td>Cost effective</td>
<td></td>
<td>Most Cost effective</td>
</tr>
</tbody>
</table>

[Links to resources](https://www.genomixlab.com/human-genome-sequencing/#.WlqEpainF3g)
GENETIC ALTERATIONS

Genomic, germline sequencing

GENETIC ALTERATIONS

Example of targeted panel in PDAC
GENETIC ALTERATIONS

Is that genetic alteration expressed?

- RNAseq evaluates transcriptome of the cell
- Consider differences between samples

**GENETIC ALTERATIONS**

**Total RNA**
- Includes rRNA, IncRNA, small RNA
- Deplete rRNA, large portion of RNA
  - Select for poly A tail
  - Selective capture
- Sequence

---

GENETIC ALTERATIONS

RNA SEQUENCING – CONSIDERATIONS

- Comparison of conditions: treated/untreated, mutant/wildtype, timecourse
- Expression changes over time or treatments
  - Predict tumor behavior, used in prognostic tests, response to drug treatment
- Can identify detrimental fusion transcripts, SNPs, alternative splicing
- Allele specific expression of targets: Is expression biased to mutation? Is there evidence of additional epigenetic regulation?
- Expression studies cannot establish if DE is the consequence or cause of the disease
EXPRESSION RESPONSE TO KRAS INDUCTION

RNAseq:
Robust downregulation
Pathway enrichment

### EXPRESSION RESPONSE TO KRAS INDUCTION

<table>
<thead>
<tr>
<th>Pathway</th>
<th>Value</th>
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<tbody>
<tr>
<td>HALLMARK_E2F_TARGETS</td>
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<tr>
<td>HALLMARK_GLYCOLYSIS</td>
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<tr>
<td>HALLMARK_BILE_ACID_METABOLISM</td>
<td>1.7</td>
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<tr>
<td>HALLMARK_ANGIOGENESIS</td>
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<td>HALLMARK_KRAS_SIGNALING_DN</td>
<td>1.8</td>
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<tr>
<td>HALLMARK_UNFOLDED_PROTEIN_RESPONSE</td>
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<td>HALLMARK_WNT_BETA_Catenin_SIGNALING</td>
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<td>HALLMARK_CHOLESTEROL_HOMEOESTASIS</td>
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<td>HALLMARK_FATTY_ACID_METABOLISM</td>
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<td>HALLMARK_NOTCH_SIGNALING</td>
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<td>HALLMARK_COMPLEMENT</td>
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<td>HALLMARK_ALLOGRAFT_REJECTION</td>
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<tr>
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<td>HALLMARK_IL2STATS_SIGNALING</td>
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<tr>
<td>HALLMARK_APOPTOSIS</td>
<td>5.6</td>
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<tr>
<td>HALLMARK_MYOGENESIS</td>
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<td>HALLMARK_TNF_A_SIGNALING_VIA_NFKB</td>
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<tr>
<td>HALLMARK_MYC_TARGETS_V2</td>
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<tr>
<td>HALLMARK_EPITHELIAL_MESENCHYAL_TRANSITION</td>
<td>20.6</td>
</tr>
</tbody>
</table>

Kerketta, et al. Manuscript in preparation 2018
EPIGENETIC REGULATION

Epigenetics: heritable changes in organisms caused by modification of gene expression rather than alteration of the genetic code itself.
EPIGENETIC REGULATION

Epigenetics
- miRNA, siRNA, piRNA, IncRNA
- DNA methylation
- Histone modifications

EXPRESSION REGULATION

https://www.researchgate.net/figure/Models-of-lncRNA-functions-Nuclear-lncRNAs-can-regulate-transcription-by-acting-as_fig1_275666853
EXPRESSION REGULATION

SMALL RNA SEQUENCING – CONSIDERATIONS

• Use unique features (size, end groups, etc) of the RNA to capture or selectively ligate adaptors, size select prior to sequencing
• Small RNA pathways are often interdependent (rely on the same proteins)
• Combining results from multiple tests – time course, mRNA transcriptome sequencing, functional assays, epigenetics, etc.
**EPIGENETIC REGULATION**

Epigenetics
- miRNA, siRNA, piRNA, IncRNA
- DNA methylation
- Histone modifications

EPIGENETIC ALTERATIONS

DNA methylation

EPIGENETIC ALTERATIONS

DNA methylation

[Diagram of DNA methylation process]
EPIGENETIC ALTERATIONS

DNA methylation

http://www.biochemj.org/content/451/1/13.figures-only
EPIGENETIC ALTERATIONS

DNA methylation

EPIGENETIC ALTERATIONS

How is DNA methylation identified and located?

- Enzymatic – cut sites sensitive or not
- Affinity – use antibodies to pull down methyl groups
- Bisulfite conversion – C becomes T

Whole Genome Bisulfite Sequencing (WGBS)
Reduced Representation Bisulfite Sequencing (RRBS)

https://www.activemotif.com/catalog/1182/ffpe-bisulfite-conversion-kit
EPIGENETIC ALTERATIONS

DNA METHYLATION SEQUENCING – CONSIDERATIONS

• Methods not 100% - Bisulfite conversion rate, pull down
• Methylation must be identified prior to amplification (not conserved)
• Combining results from multiple tests – time course, mRNA transcriptome sequencing, functional assays, epigenetics, etc.
EPIGENETIC ALTERATIONS

Example of RRBS in iKras

Differentially methylated CpGs (DMCs)

EPIGENETIC ALTERATIONS

DNA METHYLATION SEQUENCING – CANCER PRECISION MEDICINE

Michael Klutstein et al. PNAS 2017;114:9:2230-2234
EXPRESSION REGULATION

Epigenetics
• miRNA, siRNA, piRNA, IncRNA
• DNA methylation
• Histone modifications
EPIGENETIC ALTERATIONS

Chromatin dynamics, histone modifications

EPIGENETIC ALTERATIONS

Assay for Transposase-Accessible Chromatin (ATAC seq)

- Cut and ligate adapters for sequencing at regions of increased accessibility
- Euchromatin vs Heterochromatin
- Small sample size

EPIGENETIC ALTERATIONS

Chromatin dynamics, histone modifications

EPIGENETIC ALTERATIONS

• Layers of information
• Combination of histone marks, methylation
• Resulting in alteration of expression
EPIGENETIC ALTERATIONS

Chromatin Immunoprecipitation (ChIP)

- Crosslink DNA and proteins
- Fragmentation — mechanical and enzymatic (MNase)
- Antibody against histone mark or other DNA-interacting proteins
- Reverse cross link
- Comparison to input DNA

EPIGENETIC ALTERATIONS

EPIGENETIC ALTERATIONS

CHROMATIN IP SEQUENCING – CONSIDERATIONS

• Methods highly dependent on good antibodies
• Consideration of where the sequencing reads pile up
  • broad vs narrow peaks
• Combining results from multiple tests – time course, mRNA transcriptome sequencing, functional assays, epigenetics, etc.
EPIGENETIC ALTERATION UPON KRAS INDUCTION

Example of ChIP in iKras
1091 genes with expression downregulated (<-2)
Profile of H3K27Ac
EPIGENETIC ALTERATION UPON KRAS INDUCTION

Example of ChIP in iKras
389 genes with expression upregulated (>2)
Profile of H3K27Ac
EPIGENETIC ALTERATIONS

CHIP-SEQUENCING – CANCER
PRECISION MEDICINE

EPIGENETIC ALTERATIONS

Enhancer active in basal enhancers active in classical active promoters in classical

GENETIC SUMMARY

DNA alteration
- Genome, Exome DNA sequencing
- RNA sequencing
EPIGENETIC SUMMARY

Layers of control

- miRNA, siRNA, piRNA, lncRNA
- DNA methylation
- Histone modifications

OUTLINE

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SPECIAL THANKS

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