12th International VHL Medical Symposium

Boston, Massachusetts, USA

APRIL 7-9, 2016

LOCATION: Starr Center, Schepens Eye Research Institute, Boston, MA
Dear Attendees,

Welcome to the 12th International VHL Symposium in Boston, MA, organized by Massachusetts General Hospital and Boston University Medical Center and supported by the VHL Alliance.

This meeting is only possible because of the enthusiastic participation of many groups.

We are excited to have senior VHL researchers from all over US and Europe joining the meeting to discuss their latest findings.

Along with them we are proud to present young investigators that enter the field with promising enthusiasm. We are looking forward to listening to them presenting their findings.

In this year’s meeting we ventured on a new and exciting addition. We host keynote speakers that have not been traditionally working on VHL disease but their research pushes the forefront of molecular approaches in medicine; they are leaders in epigenetics, computational biology, cancer metabolism and immuno-oncology. We are looking forward to interact with them, listen to their presentations and attract them to the field! We also hope that their perspectives help expands our approach. Finally, the greatest outcome of this meeting would be the creation of new and innovative collaborations.

Lastly but most importantly, it is the VHL patients themselves that inspire the research efforts and dedication to this meeting and the work on the biology of VHL. They are here to teach us what the disease is, to share their experience, priorities and hopes with everybody in the meeting and to inspire us to continue working on curing VHL!

Welcome and enjoy the meeting!

The Meeting Organizers
Organizing Committee

Herbert T. Cohen, MD, Associate Professor of Medicine, Boston University School of Medicine

Tien Hsu, PhD, Professor of Medicine, Boston University School of Medicine; Professor, University Chair Professor, Dean, College of Health Sciences and Technology Biomedicine, Taiwan

Othon Iliopoulos, MD, Clinical Director, von Hippel-Lindau Disease/Familial Renal Cell Cancer Program, Massachusetts General Hospital Cancer Center; Associate Professor of Medicine, Harvard Medical School

Educational Grants

This activity is supported in part by educational grants from:

Agios Pharmaceuticals
Bristol-Myers Squibb
Exelisix

Sponsor

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Exhibitor

This activity is supported in part by an exhibit:

Pfizer
Educational Objectives

The 12th International VHL Medical Symposium seeks to provide physicians with an understanding of the complexities of VHL Disease and update them on the current strategies to treat VHL Disease as well sporadic RCC. These complexities are medical and social; not only the therapeutic targets for VHL-related tumors are limited but each individual VHL patient faces a lifetime long battle with tumors arising in multiple organs. As such, VHL is therefore a multi-specialty disease. The symposium is adopted a multidisciplinary approach to understanding and treating VHL Disease and sporadic RCC, as evidenced by the integrated presentation from, basic researchers, translational researchers, clinical researchers, healthcare professionals and VHL patients and their families. Each groups of speakers and/or participants will contribute to the important discussion about providing excellent and comprehensive care while working towards the ultimate goal of finding a cure.

At the conclusion of this activity, participants will be able to:

1. Identify the major signaling pathways that participate in the initiation and growth of VHL-related tumors.

2. Describe how deregulation of basic cellular mechanisms that contribute to cell transformation upon VHL loss.

3. Identify validated and emerging targets for treatment of VHL disease and sporadic RCC.

4. Describe the strengths and weakness of each animal model of VHL disease and/or RCC, available to the scientific community today and how these models can be used to screen for or to validate novel therapeutic approaches for VHL disease and/or sporadic RCC.

5. Recognize how the tumor microenvironment contributes to tumor development in VHL-related tumors and sporadic RCC, including the role of microbiome as a disease modifier.

6. Identify the basic mechanisms of tumor escape from immune surveillance and how they can be manipulated therapeutically for tumor immunotherapy.

7. Summarize the current role of RTK inhibitors in treating VHL-related disease and RCC.

8. Examine the complex psychological burden that VHL disease generates for the patients and their families, the role of chronic disease in psychosocial dynamics and the strategies to build a healthy lifestyle while facing a long life battle with cancer.

9. Apply appropriate surveillance practices in relation specialized disease centers and patient initiated databases.
Accreditation Statement

This activity has been planned and implemented in accordance with the accreditation requirements and policies of the Accreditation Council for Continuing Medical Education (ACCME) through the joint providership of Boston University School of Medicine and the VHL Alliance. Boston University School of Medicine is accredited by the ACCME to provide continuing medical education for physicians.

Boston University School of Medicine designates this live activity for a maximum of 18.50 AMA PRA Category 1 Credits™. Physicians should only claim credit commensurate with the extent of their participation in the activity.

Disclosures

Boston University School of Medicine asks all individuals involved in the development and presentation of Continuing Medical Education (CME) activities to disclose all relationships with commercial interests. This information is disclosed to CME activity participants. Boston University School of Medicine has procedures to resolve any apparent conflicts of interest. In addition, faculty members are asked to disclose when any unapproved use of pharmaceuticals and devices is being discussed.

James Brugarolas MD, PhD: Consultant at Sandoz, Bethyl Advisory Board, Novartis Advisory Board
Ralph J. DeBerardinis, MD, PhD: Consultant, Stockholder, and Scientific Advisory Board at Agios Pharmaceuticals, Consultant, Stockholder, and Scientific Advisory Board at Peloton Therapeutics
Koen Dreijerink MD, PLD: Consultant at Eisai
Gordon Freeman PhD: DFCI administered patent licensing fees/pending royalties at Roche, Bristol Myers Squibb, Merck, and Novartis; Consultant at Roche, Bristol Myers Squibb, Eli Lilly, Surface Oncology, Bethyl Laboratories, Seattle Genetics, Genocea Biosciences; Grant/Research Support from Roche, Bristol Myers Squibb, Novartis
Amato J. Giaccia PhD: Stockholder at RUGA BoiTech
Rachel Giles MD, PhD: Grant/Research Support from Bristol-Meyers Squibb, Grant/Research Support and Consultant at Novartis Oncology, Grant/Research Support and Consultant at Pfizer, Grant/Research Support from Bayer
Pascal Hammel MD: Grant/Research Support, Consultant, and Speakers Bureau at Celgene, Consultant at Merck Serono, Consultant at Ipsen, Consultant at AstraZeneca, Grant/Research Support at Roche
Eric Jonasch MD: Consultant at Bristol-Meyers Squibb, Grant/Research Support from Exelixis; Grant/Research Support and Consultant at GlaxoSmithKline, Grant/Research Support and Consultant at Novartis, Grant/Research Support and Consultant at Pfizer
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Bruce R. Korf MD, PhD: Grant/Research Support from Novartis, Consultant at Accolade, Speaker at Sequenom, Consultant at AstraZeneca
Marcela Valderrama Maus MD, PhD: Grant/Research Support and Consultant at Novartis, Consultant at Neon
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Eli M. Wallace PhD: Employee and Stockholder at Peloton Therapeutics, Inc.
Johnathan R. Whetsine, PhD: Consultant at Qsonica and Celgene

The following have nothing to disclose:
Peter Bakke; Andrea Berkemeier; Barbara Bezemer; Kivanc Birsoy PhD; Herbert T. Cohen MD; Maria F. Czyzyk-Krzeska MD, PhD; Ruhee Dere PhD; Tobias Else MD; Ian Frew MD, PhD; Raymon H. Grogan MD; Scott M. Haake MD; Julian Hess; Tien Hsu PhD; Othon Iliopoulos MD, PhD; Jacques Izard PhD; Robin Kadlecik; Chan-Yen Kuo PhD; Mehdi Mallapor PhD; Ana Marguerita Martins; Metelo MSc; Eijiro Nakamura MD, PhD; Channing J. Paller MD; Daniel Segal PhD; Ilene Sussman PhD; Karl Wallace; Anna Waller; Wenyi Wei PhD; Halfeng Yang PhD; Qing Zang PhD

Unlabeled/Investigational use:
Dr. Ruhee Dere, Dr. Othon Iliopoulos, Dr. Tien Hsu and Dr. Gordon Freeman will be discussing unlabeled/investigational uses of a commercial product.

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### DAY 1: THURSDAY, APRIL 7, 2016

**SESSION TITLE: CELL BIOLOGY AND SIGNALING**

**MODERATOR: OTHON ILIOPOULOS, MD**

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<tr>
<th>Time</th>
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<th>Speaker</th>
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<tr>
<td>8:00 AM</td>
<td>WELCOME</td>
<td>Herbert T. Cohen MD, Tien Hsu, PhD, Othon Iliopoulos, MD, Ilene Sussman, PhD</td>
</tr>
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</table>
| 8:15 AM | KEYNOTE: Targeted Editing of the Genome                                         | J. Keith Juong, MD, PhD

  Professor of Pathology, Harvard Medical School; Associate Pathologist, Associate Chief for Research, The Jim and Ann Orr MGH Research Scholar, Massachusetts General Hospital |
| 9:15 AM | Jade-1 as pVHL Target of pVHL in Renal Cancer                                  | Herbert T. Cohen, MD

  Associate Professor of Medicine, Boston University School of Medicine |
| 9:40 AM | pVHL Suppresses Akt Kinase Activity and Oncogenic Function in a Proline-Hydroxylation Dependent Manner | Wenyi Wei, PhD

  Associate Professor of Medicine, Harvard Medical School |
| 10:05 AM | Molecular Functions of VHL-Regulated LC3C-Dependent Autophagic Program        | Maria Czyzyk-Krzeska, MD, PhD

  Professor, University of Cincinnati Cancer Center |
| 10:30 AM | BREAK                                                                          | Fraydoon Rastinejad, PhD

  Professor, Sanford Burnham Prebys Medical Discovery Institute |
| 10:40 AM | Structures and Drug Binding Potentials of HIF-alpha/ARNT Complexes            | Ruhee Dere, PhD

  Texas A&M Health Science Center |
| 11:05 AM | Proffered Paper: Von Hippel-Lindau Tumor Suppressor Mediates Aurora Kinase A Degradation to Regulate Ciliogenesis | Qing Zhang, PhD

  The University of North Carolina at Chapel Hill, Lineberger Comprehensive Cancer Center |
| 11:20 AM | Proffered Paper: ZHX2 as a Potential pVHL Target in Kidney Cancer             | Qing Zhang, PhD

  The University of North Carolina at Chapel Hill, Lineberger Comprehensive Cancer Center |
## DAY 1: THURSDAY, APRIL 7, 2016

### SESSION TITLE: SYSTEMS BIOLOGY
**MODERATOR: AMATO J. GIACCIA, PHD**

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<tr>
<th>Time</th>
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<tr>
<td>11:35 AM</td>
<td>Identification of Oncogenic Drivers by Genomic Analysis of Tumor Samples</td>
<td>Julian Hess, Computational Biology, Broad Institute of Harvard and Massachusetts Institute of Technology</td>
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<tr>
<td>12:15 PM</td>
<td>Comprehensive Molecular Characterization of Renal Cell Carcinoma: The TCGA Experience</td>
<td>Scott M. Haake, MD, Instructor in Medicine, Vanderbilt University School of Medicine</td>
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<tr>
<td>12:40 PM</td>
<td>Metabolic Targets in RCC</td>
<td>Othon Iliopoulos, MD, Clinical Director, von Hippel-Lindau Disease/Familial Renal Cell Cancer Program, Massachusetts General Hospital Cancer Center; Associate Professor of Medicine, Harvard Medical School</td>
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<td>1:05 PM</td>
<td>LUNCH / POSTER SESSION</td>
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<tr>
<td>2:05 PM</td>
<td>Epigenetics Provides Insights into Copy Number Heterogeneity, Drug Resistant Gene Selection and Therapeutic Response</td>
<td>Johnathan R. Whetstine, PhD, Associate Professor, Medicine Harvard Medical School</td>
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<tr>
<td>2:30 PM</td>
<td>Proffered Paper: Mps1 Mediated Phosphorylation of Hsp90 Confers RCC Sensitivity and Selectivity to Hsp90-Drugs</td>
<td>Mehdi Mollapour, PhD, SUNY Upstate Medical University</td>
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<tr>
<td>2:45 PM</td>
<td>Proffered Paper: The Tumor Suppressor Functions of PBRM1 in Kidney Cancer</td>
<td>Haifeng Yang, PhD, Thomas Jefferson University</td>
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### SESSION TITLE: ANIMAL MODELS
**MODERATOR: DANIEL SEGAL, PHD**

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<tr>
<td>3:00 PM</td>
<td>Inhibition of miRNA-132/212 Suppresses VHL-Regulated Pathological Angiogenesis in Zebrafish and Renal Cell Carcinoma</td>
<td>Rachel Giles, MD, PhD, Associate Professor Department of Nephrology and Hypertension, UMC Utrecht</td>
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<tr>
<td>3:25 PM</td>
<td>Generation of a Mouse Model of Clear Cell Renal Cell Carcinoma</td>
<td>Ian Frew, PhD, Assistant Professor, Institute of Physiology, University of Zurich</td>
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<td>3:50 PM</td>
<td>BREAK</td>
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<tr>
<td>4:00 PM</td>
<td>Arginine—a Potent Refolder of Mutant pVH—a Possible Therapeutic for the VHL Syndrome</td>
<td>Daniel Segal, PhD, Professor, Department of Molecular Microbiology &amp; Biotechnology, Tel Aviv University</td>
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### DAY 1: THURSDAY, APRIL 7, 2016

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<tr>
<td>4:25 PM</td>
<td>RCC: Molecular Pathways and Novel Therapies</td>
<td>James Brugarolas, MD, PhD</td>
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<td>Kidney Cancer Program Leader, Associate Professor, Internal Medicine, Simmons Comprehensive Cancer Center, UT Southwestern Medical Center</td>
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<tr>
<td>4:50 PM</td>
<td>Proffered Paper: Establishment of Novel Models of VHL Disease by Using Patient-Derived Induced Pluripotent Stem Cells</td>
<td>Eijiro Nakamura MD, PhD</td>
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<td>Kyoto University Graduate School of Medicine</td>
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**Symposium Dinner at the Wyndham Boston Beacon Hill**

### DAY 2: FRIDAY, APRIL 8, 2016

**SESSION TITLE: CANCER METABOLISM**  
**MODERATOR: ERIC JONASCH, MD**

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<tr>
<td>8:00 AM</td>
<td>KEYNOTE: Systems Approach to Metabolism</td>
<td>Ralph J. DeBerardinis, MD, PhD</td>
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<td>Sowell Family Scholar in Medical Research, Assistant Professor of Pediatrics and Genetics, University of Texas—Southwestern Medical Center</td>
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<tr>
<td>9:00 AM</td>
<td>The Role of PGC-1 alpha in RCC</td>
<td>Amato J. Giaccia, PhD</td>
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<td>Jack, Lulu and Sam Willson Professor, Professor of Radiation Oncology, and by courtesy, of Obstetrics and Gynecology and of Surgery, Stanford School of Medicine</td>
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<tr>
<td>9:25 AM</td>
<td>Systematic Approaches to Study Cancer Cell Metabolism</td>
<td>Kivanc Birsoy, PhD</td>
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<td>Assistant Professor, Head, Laboratory of Metabolic Regulation and Genetics, The Rockefeller University</td>
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<tr>
<td>9:50 AM</td>
<td>Proffered Paper: ER Stress in von Hippel-Lindau Tumor Suppressor Gene Mutant Kidney Cells and the Induction of Inflammatory Response</td>
<td>Chan-Yen Kuo, PhD</td>
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<td>Institute of Systems Biology and Bioinformatics, National Central University</td>
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<td>10:05 AM</td>
<td>BREAK</td>
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### SESSION TITLE: IMMUNOLOGY AND TUMOR MICROENVIRONMENT
**MODERATOR: TIENT HSU, MD**

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<tr>
<td>10:15 AM</td>
<td><strong>KEYNOTE:</strong> Role of Co-Stimulatory Molecules and Immune Cell Checkpoints in Cancer Suppression</td>
<td>Gordon J. Freeman, PhD Dana-Farber Cancer Institute; Professor, Medicine, Harvard Medical School</td>
</tr>
<tr>
<td>11:15 AM</td>
<td>Engineering T cells to Target Cancer</td>
<td>Marcela Valderrama Maus, MD, PhD Assistant Professor of Medicine, Director of Cellular Immunotherapy, Massachusetts General Hospital</td>
</tr>
<tr>
<td>11:40 AM</td>
<td>ER Stress and Inflammatory Response in VHL Inactivated Kidney Tissue</td>
<td>Tien Hsu, PhD Professor of Medicine, Boston University School of Medicine; Professor, University Chair Professor, Dean, College of Health Sciences and Technology Biomedicine, Taiwan</td>
</tr>
<tr>
<td>12:05 PM</td>
<td>Proffered Paper: Identification of Novel Oncogenic Pathways in Central Nervous System Hemangioblastomas</td>
<td>Ana Marguerita Martins Metelo, MS Massachusetts General Hospital</td>
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<td>12:20 PM</td>
<td>LUNCH / POSTER SESSION</td>
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### SESSION TITLE: THERAPEUTIC CHALLENGES
**MODERATOR: HERBERT T. COHEN, MD**

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<tr>
<td>1:20 PM</td>
<td>Targeting VHL Tumors with RTK Inhibitors</td>
<td>Eric Jonasch, MD Professor, Department of Genitourinary Medical Oncology, Division of Cancer Medicine, The University of Texas, MD Anderson Cancer Center</td>
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<tr>
<td>1:45 PM</td>
<td>Microbiome Association with Cancer</td>
<td>Jacques Izard, PhD Harvard School of Dental Medicine</td>
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<td>2:10 PM</td>
<td>Early Data from VHLA’s Patient Databank</td>
<td>Ilene Sussman, PhD Executive Director, VHL Alliance</td>
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### DAY 2: FRIDAY, APRIL 8, 2016

**SESSION TITLE: CLINICAL APPLICATION**  
**MODERATOR: RACHEL GILES, MD, PHD**

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<tr>
<td>3:05 PM</td>
<td>VHL and Family Planning: Ethical Considerations and the Importance of Counseling</td>
<td>Andrea Berkemeier</td>
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</table>
| 3:20 PM | KEYNOTE: Integrated Care for Cancer Patients                                  | Channing J. Paller, MD  
Assistant Professor of Oncology, Sidney Kimmel Comprehensive Cancer Center |
| 4:20 PM | Panel Discussion: VHL Clinical Care Centers  
Representatives from:  
- Koen Dreijerink, MD, PLD, Internist-Endocrinologist University Hospital Utrecht  
- Pascal Hammel, MD, PhD, Gastroenterologist, VHL Clinical Care Center and French NCI Expert National Center “PREDIR”  
- Bruce R. Korf, MD, PhD, Wayne H. and Sara Crews Finley Chair in Medical Genetics, Professor and Chair, Department of Genetics, Director, Heflin Center for Genomic Sciences, University of Alabama at Birmingham  
- Raymon H. Grogan, MD, Assistant Professor of Surgery; Director, Endocrine Surgery Research Program, University of Chicago  
- Tobias Else, MD, Assistant Professor, Internal Medicine University of Michigan |                                                                 |
| 5:25 PM | ADJOURN                                                                        |                                                                                |
### DAY 3: SATURDAY, APRIL 9, 2016

**SESSION TITLE: The Patient’s Perspective: What is Behind the Monitoring and Surgeries?**  
**MODERATOR: SUZANNE NYLANDER, OD**

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<th>Speaker</th>
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| 9:00 AM  | WELCOME                                                     | Ilene Sussman, PhD  
Executive Director, VHL Alliance                                         |
| 9:10 AM  | Learning from Other Cancer Research                         | Othon Iliopoulos, MD  
Clinical Director, von Hippel-Lindau Disease/Familial Renal Cell Cancer Program, Massachusetts General Hospital Cancer Center; Associate Professor of Medicine, Harvard Medical School |
| 9:40 AM  | Highlights of Current VHL Research                          | Rachel Giles, PhD  
Associate Professor Department of Nephrology and Hypertension, UMC Utrecht |
| 10:10 AM | What the Future Might Hold                                  | Eric Jonasch, MD  
Professor, Department of Genitourinary Medical Oncology, Division of Cancer Medicine, The University of Texas, MD Anderson Cancer Center |
| 10:40 AM | BREAK                                                       |                                                                        |
| 10:55 AM | Adding an Additional Challenge to the Teen Age Years        | Karli W, invited                                                       |
| 11:05 AM | Challenges of the Young Adult                              | Peter B                                                                |
| 11:20 AM | The Importance of Counseling                                | Andrea B                                                               |
| 11:30 AM | The Advantages of Meeting                                   | Barbara B                                                              |
| 11:50 AM | **DISCUSSION: How Can We Work Together to Reduce the Feelings of Isolation?** |                                                                        |
| 12:10 PM | The Impact on the Caregiver: Parent Perspective             | Anna W                                                                 |
| 12:20 PM | The Impact on the Caregiver: Spouse Perspective             | Robin K                                                                |
| 12:20 PM | **DISCUSSION: How Can We Meet the Needs of Caregivers?**    |                                                                        |
| 1:10 PM  | LUNCH                                                       |                                                                        |
Presentation Abstracts
SESSION TITLE
Cell Biology and Signaling
KEYNOTE ADDRESS

Targeted Editing of the Genome

J. Keith Joung
Department of Pathology, Harvard Medical School; Massachusetts General Hospital, Boston, MA, USA

CRISPR-Cas nucleases are widely used for a wide range of research applications and are being developed for use as therapeutic agents. In this talk, I will provide a general introduction to CRISPR-Cas nucleases and then discuss our recent work to define, optimize, and alter the specificities of these reagents in human cells. Our studies provide highly sensitive, genome-wide, unbiased methods for defining nuclease specificities, reduce genome-wide off-target effects to undetectable levels, and increase the targeting range of CRISPR-Cas nucleases. These advances provide information and reagents that will broaden the use of CRISPR-Cas nucleases.
Jade-1 as a Target of pVHL in Renal Cancer

Delia Lopez1,2, Ruoyu Tian1,4, Mame B. Basse1,2, Deepthi Desai1,2, Neil Lajkiewicz5, Lauren E. Brown5, John A. Porco5, Amit K. Mittal1,2, Ajit Bharti3, Rachel L. Flynn2,3, Herbert T. Cohen1,4

1Renal Section, Departments of 2Medicine, 3Pharmacology, and 4Pathology, Boston University School of Medicine and Boston Medical Center; 5The Center for Chemical Methodology and Library Development, Boston University, Boston, MA, USA

Clear-cell renal-cell carcinoma (ccRCC) is a major clinical challenge worldwide and also a devastating manifestation of von Hippel-Lindau (VHL) disease. In 90% of cases, ccRCC is initiated by loss of the tumor suppressor pVHL. In order to better understand ccRCC pathogenesis and develop new therapies, we screened for novel, growth suppressive pVHL interactors and identified the multifunctional zinc-finger, plant homeodomain protein Jade-1 as a pVHL partner and the first member of the Jade family of proteins. Intriguingly, pVHL stabilizes Jade-1 protein and, as might be expected, Jade-1 exhibits consistently growth suppressive biology and biochemistry. We have shown that Jade-1 is a pVHL effector in kidney cancer and tumor suppressor itself. Others have shown that Jade-2 may be a tumor suppressor in neuroblastoma.

Genome instability is a hallmark of solid tumors, including ccRCC, and DNA repair defects may be exploited as part of a rational approach to cancer therapy. Most recently, our coimmunoprecipitation studies have found that Jade-1 associates with many DNA repair proteins in kidney cells, suggesting Jade-1 is directly involved in DNA double-strand break repair, mismatch repair and nucleotide excision repair. In response to DNA damage, Jade-1 is inducible, translocates to the nucleus and highlights nuclear speckles that may be sites of active DNA repair. In colony forming assays, we have found that silencing JADE1 affects cell survival in response to DNA damage, further supporting its role in the DNA repair process. Thus, Jade-1 is a promising new player as tumor suppressor and DNA repair protein.

We have also been studying Jade-1 protein fate. Jade-1 is short lived and degraded by the proteasome pathway. Proteasome inhibitors in current clinical use for multiple myeloma dramatically increase levels of endogenous Jade-1 protein, suggesting that growth-suppressive effects of these agents may be mediated in part by tumor suppressor Jade-1. We have recently identified a ubiquitin ligase for Jade-1 as Siah-1. In order to develop more specific small molecule inhibitors of the proteasome and of the interaction between Jade-1 and its ubiquitin ligases, we have generated a high-throughput screening assay and identified several classes of small molecule inducers of Jade-1 protein abundance. Thus, by pharmacologically modulating Jade-1 protein levels, we may be able to inhibit cell growth and alter specific DNA repair mechanisms. Such agents may have clinical utility in ccRCC and other settings.
pVHL Suppresses Akt Kinase Activity and Oncogenic Function in a Proline-Hydroxylation Dependent Manner

Jianping Guo¹, Abhishek A. Chakraborty², Pengda Liu¹, Wenjian Gan¹, Xingnan Zheng³, Jin Q. Cheng⁴, Alex Toker², Qing Zhang³, John M. Asara⁵, William G. Kaelin, Jr.²,⁶,⁷, Wenyi Wei¹,⁷

¹Department of Pathology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA; ²Dana-Farber Cancer Institute and Brigham and Women’s Hospital, Harvard Medical School, Boston, MA, USA; ³Lineberger Comprehensive Cancer Center, University of North Carolina School of Medicine, Chapel Hill, NC, USA; ⁴H. Lee Moffitt Cancer Center and Research Institute, Tampa FL, USA; ⁵Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA; ⁶Howard Hughes Medical Institute, Chevy Chase, MD, USA

Mammalian/mechanistic target of rapamycin (mTOR) kinase inhibitors are widely used to treat advanced renal carcinomas harboring frequent genetic mutations in the von-Hippel-Lindau (VHL) tumor suppressor, but it remains poorly defined whether pVHL governs Akt activity. Here, we report that the serine/threonine protein kinase Akt undergoes proline-hydroxylation that is catalyzed by the EglN1 prolyl-hydroxylase. pVHL directly binds to and inhibits Akt kinase activity in a proline-hydroxylation-dependent manner. Pathophysiologically, under hypoxic conditions, or in type 1 VHL-deficient renal carcinomas, Akt is aberrantly activated due to deficiencies in association with, and subsequent inhibition by pVHL, thereby promoting cell survival and tumorigenesis. Cancer-associated mutations in Akt, including Akt1-G311D or Akt2-P127N, are impaired in hydroxylation and escape subsequent inhibition by pVHL, leading to hyper-activation of Akt oncogenic function. Since most solid tumors thrive under hypoxic conditions, these findings provide molecular insight into mechanisms leading to aberrant activation of Akt oncogenic signaling observed in most tumor settings.
Molecular Functions of VHL-Regulated LC3C-Dependent Autophagic Program

Maria F. Czyzyk-Krzeska¹, Adam D. Price¹, Megan E. Bischoff¹, Birgit Ehmer¹, Johnston Chu¹, Jarek Meller², David R. Plas¹

¹Department of Cancer Biology, ²Department of Environmental Health, University of Cincinnati College of Medicine, Cincinnati, OH, USA

Objective: To identify specific function of a VHL-regulated autophagic program depending on MAP1LC3C (LC3C).

Introduction/Background: Autophagy is a homeostatic function by which cells process their own organelles and proteins to eliminate defective molecules and to recycle nutrients. Many cancers become addicted to autophagy as a source of nutrients. In human cells, there are three MAP1LC3 (LC3) paralogs, LC3A, LC3B, and LC3C. LC3A and LC3B are very similar and co-localize on autophagosomes. In contrast, LC3C is only 60% similar to LC3A/B, has additional N- and C-terminal peptides and forms autophagosome vesicles independent from LC3A/B. Our laboratory demonstrated that VHL regulates autophagy and that one of the mechanisms regulating autophagic specificity in RCC involves utilization of different LC3s. VHL inhibits LC3B autophagy through direct and indirect effects of miR-204, but induces LC3C-autophagy, in a mechanism involving inhibition of HIF. Importantly, while LC3B-mediated autophagy supports tumor growth, LC3C-dependent autophagy has tumor suppressing activity in ccRCC. Currently, the biological activity of LC3C is not understood.

Results and Methods: Under serum starvation conditions, LC3C, but not LC3B, colocalizes with postmitotic midbody rings (PMMBR) and targets them for autophagosomal degradation. Degradation of PMMBR by LC3C requires LC3C C-terminal peptide. Midbody is a structure generated during final stage of cytokinesis in the intercellular bridge and its formation is necessary for the membrane severance. However, after successful cytokinesis, the midbody is either asymmetrically inherited by one of the daughter cells, where it can be either maintained or undergoes intracellular autophagic degradation, or it is released to the extracellular environment. Cancer cells maintain larger numbers of PMMBR, and recently PMMBR have been implicated as scaffolds regulating stem cell fate and proliferation. Consistently, cells with LC3C-knockdown have higher number of PMMBR per cell, and when dividing, the preexisting midbodies are inherited asymmetrically by one of the daughter cells. Reexpression of the wild type LC3C leads to the recovery of symmetric cell divisions with only one midbody formed during cytokinesis.

We also found that PMMBR are often positioned in the caveolae, plasma membrane invaginations containing caveolin (CAV1) and cholesterol. LC3C targets for lysosomal degradation CAV-1, but not the other lipid raft protein, flotillin and LC3C-positive autophagosome stain for both, PMMBR marker, MKLP1, and CAV1. Importantly, CAV-1 has been shown by us and others to act as an oncogene in renal cancer. However, the molecular mechanism through which CAV1 supports growth of ccRCC is currently not well understood. The data implicate interactions between PMMBRs and caveolin in renal oncogenesis.

Conclusions: Our working hypothesis is that positioning of PMMBR in caveolin raft creates a scaffold which regulates an oncogenic signaling pathway. LC3C-dependent autophagy exercises its tumor suppressing activity by targeting this pathway for degradation at multiple steps.
Hypoxia in solid tumors contributes to aggressive phenotypes and is responsible for the failure of many current therapies. The hypoxia-inducible factors (HIFs) are versatile transcription factors that mediate the progression of hypoxic tumors. HIF-regulated programs drive erythropoiesis, angiogenesis, and the metabolic switch to glucose utilization. These adaptations enhance energy utilization, drug efflux, cell proliferation, and anti-apoptosis pathways that promote tumor growth and drug resistance. Because many classic chemotherapeutic agents fail to impact hypoxic cancers, novel strategies are needed to target tumor growth under hypoxic conditions. The HIF-α proteins belong to a common mammalian basic helix-loop-helix-period-ARNT-single minded (bHLH-PAS) family that further includes their common heterodimerization partner: aryl hydrocarbon receptor nuclear translocator (ARNT). Transcriptionally active HIFs consist of heterodimers of each HIF-α subunit with the commonly shared ARNT (HIF-1β) subunit. Here we describe crystal structures for both HIF-2α:ARNT and HIF-1α:ARNT heterodimers in complexes that include a series of bound small-molecules and their hypoxia response element DNA. Our detailed crystallographic observations reveal five distinct small-molecule pockets within each of HIF-2α:ARNT and HIF-1α:ARNT heterodimers. These newly identified ligand pockets are positioned inside each of the four individual PAS domains of heterodimers, with the fifth site forming through subunit heterodimerization. The five pockets indicate it may be possible to identify a wide variety of small-molecule drugs that can inhibit HIF functions for anti-cancer therapy. We additionally examined a series HIF-α mutations linked to human cancers, and found these sites to map to sensitive locations responsible for DNA-binding, the integrity of PAS pockets, and subunit heterodimerization with ARNT.
PROFFERED PAPER:
von Hippel Lindau Tumor Suppressor Mediates Aurora Kinase A Degradation to Regulate Ciliogenesis

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Loss of heterozygosity at the von Hippel-Lindau (VHL) tumor suppressor gene locus on chromosome 3p is commonly associated with renal cell carcinoma (RCC). VHL is an E3 ligase, most famed for its role in proteasome-mediated degradation of hypoxia inducible factor α (HIFα), although more recently VHL has been implicated in numerous non-HIF related functions, including stabilization of microtubules. Consistent with VHL’s role in microtubule stabilization, VHL loss results in loss of the primary cilium, and the development of renal cysts and tumors, categorizing VHL disease as a ciliopathy. Aurora Kinase A (AURKA), in a novel non-mitotic role was identified as an upstream kinase of histone deacetylase 6 (HDAC6) whose activation caused disassembly of the primary cilium. In this study, we show that AURKA is a novel target for VHL’s E3-ligase activity. VHL directly regulates AURKA expression by promoting AURKA degradation via the 26S proteasome. In vitro and in vivo ubiquitination assays showed enhanced AURKA ubiquitination in the presence of VHL. We found that VHL directly ubiquitinates AURKA via a multi-mono ubiquitin chain linkage, in contrast to the more traditional and abundant K48-linkage of proteins targeted for proteasome-mediated degradation. Biochemical studies revealed that unlike HIF1α recognition and degradation by VHL, which requires prolyl hydroxylating, VHL interacted with, and degraded AURKA independent of its hydroxylating status, suggesting an alternate recognition motif on AURKA. Importantly, we observed increased AURKA ubiquitination in cells induced to ciliate, suggesting that VHL degradation of AURKA potentially modulates primary cilia formation by blocking the cilia disassembly pathway. Pharmacologic inhibition of AURKA and HDAC6 restored the ability of cells with an acute loss of VHL to form primary cilia. Thus, our data identify a novel target of VHL’s E3 ligase activity that can be therapeutically targeted to rescue the ciliary defect associated with VHL-null renal cell carcinoma.
PROFFERED PAPER:
ZHXX2 as a Potential pVHL Target in Kidney Cancer

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Objectives: To identify potential pVHL substrates and characterize their function in kidney tumorigenesis.

Introduction/Background: Loss of VHL-encoded protein (pVHL) function/expression leads to stabilization of a set of proteins regulating its downstream signaling, which have been found to contribute substantially to the transforming phenotype of kidney cancer. It is critical to identify pathways that are affected by pVHL loss and that are key contributors to the overall kidney cancer program, which will help design therapeutic invention strategies to target these drivers for kidney cancer. Although degradation of HIF-α is important for VHL’s tumor suppressor function, several lines of evidence suggest that additional pVHL substrates exist. However, the identification of novel and relevant pVHL E3 ligase complex substrates remains a daunting challenge. Here we mainly address this challenge, which will likely open new therapeutic avenues in targeting kidney cancer.

Methods: We employed a genome-wide in vitro expression strategy coupled with GST-binding screening and identified ZHX2 as a potential pVHL target. In addition, we examined the effect of ZHX2 on kidney cancer cell proliferation, soft agar growth, invasion and orthotopic tumor growth. Mechanistically, we performed microarray and ChIP-seq to identify critical ZHX2 downstream target genes that may mediate its effect on kidney tumorigenesis.

Results: We identified that ZHX2 as a potential pVHL target. ZHX2 hydroxylation at least partially mediates its protein stability regulation by pVHL. Depletion of ZHX2 in several kidney cancer cells decreases their cell proliferation, soft agar growth, invasion as well as orthotopic tumor growth. By performing integrated analyses of ZHX2 ChIP-Seq and microarray, we identified a subset of NF-kB target gene being positively regulated by ZHX2. Mechanistically, we show that ZHX2 binds with RelA/p65 and mediates its translocation from cytoplasm to nucleus, where p65 binds to a subset of NF-kB target gene promoters.

Conclusions: Our research established that ZHX2 as a potential pVHL target that plays an important role in kidney tumorigenesis. In addition, ZHX2 may act as a mechanistic link between pVHL loss and hyperactivation of NF-κB in kidney cancer.
SESSION TITLE
Systems Biology
Identification of Oncogenic Drivers by Genomic Analysis of Tumor Samples

Julian Hess
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Although a few cancer genes are mutated in a high proportion of tumors of a given type (>20%), most are mutated at intermediate frequencies (2–20%). The exponentially falling cost of genomic sequencing has allowed us to assemble cohorts large enough to have the statistical power to identify some of these lower frequency genes. In this talk, we will examine statistical methods we have developed for accurately discriminating driver mutations from passenger mutations, discuss novel cancer gene candidates our methods have identified, and conclude with an analysis on how many more patients must be sequenced to yield enough statistical power to identify the majority of cancer genes.
Comprehensive Molecular Characterization of Renal Cell Carcinoma: The TCGA Experience

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The vast majority of adult kidney cancer originates from the outer layer or cortex of the kidney and are termed renal cell carcinomas. Renal cell carcinomas are a heterogeneous group of diseases with distinct histologies. Independent genetic syndromes predispose patients to the development of these distinct histologic subtypes and provided the first clues regarding their distinct molecular biology. Comprehensive molecular characterization of three major renal cell carcinoma subtypes (clear cell, papillary, and chromophobe) has been completed through the intense, coordinated efforts of The Cancer Genome Atlas Research Network. This effort included analysis of >600 tumors with multiple analytical platforms including whole-exome sequencing, mRNA and miRNA sequencing, copy number analysis, DNA methylation analysis, and proteomic analysis. Common themes emerged across the subtypes including metabolic reprogramming and mutations in chromatin regulators. This systematic analysis of hundreds of tumors across the renal cell carcinoma spectrum has further resolved the core molecular biology of these distinct yet related cancers.
Metabolic Targets in RCC

Othon Iliopoulos

von Hippel-Lindau Disease/Familial Renal Cell Cancer Program, Massachusetts General Hospital Cancer Center, Boston, MA; Co-Leader in Kidney Cancer and Member of Cancer Genetics, Harvard Medical School, Boston, MA, USA

VHL-/− renal cell carcinoma (RCC) cells use glutamine to generate citrate and lipids through reductive carboxylation (RC) of α-ketoglutarate (αKG). In addition to being a source of lipogenic acetyl-CoA, glutamine can generate aspartate – the carbon source for pyrimidine biosynthesis and glutathione (GSH) – the key metabolite for redox balance. Here we showed that VHL-/− RCC cells rely on RC-derived aspartate to maintain de novo pyrimidine biosynthesis. Glutaminase 1 (GLS1) inhibitors depleted pyrimidines in VHL-/− cells but not in VHL+/+ cells, which utilized glucose oxidation for aspartate production. GLS1 inhibitors also increased reactive oxygen species (ROS) in VHL-/− cells through GSH biosynthesis repression. GLS1-inhibitor induced nucleoside depletion and ROS enhancement activated an intra-S-phase checkpoint, corresponding to DNA replication stress and suppressed S-phase transition and growth of VHL-/− RCC cells. DNA replication stress and cell growth were rescued by administration of glutamate or αKG and partially rescued by supplementation of nucleobases or N-acetyl cysteine. Lastly, we showed that the PARP inhibitor olaparib synergizes with GLS1 inhibitors in suppressing growth of VHL-/− cells in vitro and in vivo. These mechanistic insights will facilitate the development of novel therapeutic strategies for RCC.
Epigenetics Provides Insights into Copy Number Heterogeneity, Drug Resistant Gene Selection and Therapeutic Response

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Acquired somatic copy number alterations are a feature of drug resistant tumors; however, the mechanistic basis for their occurrence remains unclear. We have recently identified the histone tri-demethylase KDM4A as the first enzyme capable of promoting site-specific copy number changes. KDM4A overexpression promotes localized copy gain without global chromosome instability. Tumors with increased KDM4A levels are enriched in copy gains for cytobands observed in cell culture models. We further demonstrate that these events are the result of rereplication. We have now established that additional epigenetic mechanisms are influencing copy gains in the human genome. Based on these data we are interrogating the druggability of these processes. We further demonstrated that physiological triggers and microRNA networks are involved in modulating copy gains and drug resistant gene expression. Taken together, we have identified genetic, epigenetic and environmental factors promoting copy number heterogeneity in tumors and established that these events are targetable through inhibition of chromatin regulators.
PROFFERED PAPER:
Mps1 Mediated Phosphorylation of Hsp90 Confers RCC Sensitivity and Selectivity to Hsp90-Drugs

Mark R. Woodford\textsuperscript{1,2,3}, Diana M. Dunn\textsuperscript{1,2,3}, Dimitra Bourboulia\textsuperscript{1,2,3}, Gennady Bratslavsky\textsuperscript{1,3}, Mehdi Mollapour\textsuperscript{1,2,3}

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The molecular chaperone Heat Shock Protein-90 (Hsp90) protects deregulated signaling proteins that are vital for tumor growth and survival. Tumors generally display sensitivity and selectivity towards Hsp90 inhibitors; however, the molecular mechanism underlying this phenotype remains undefined. We report that the mitotic checkpoint kinase Mps1 phosphorylates a conserved threonine residue in the amino-domain of Hsp90. This, in turn, regulates chaperone function by reducing Hsp90 ATPase activity while fostering Hsp90 association with kinase clients, including Mps1. Phosphorylation of Hsp90 is also essential for the mitotic checkpoint because it confers Mps1 stability and activity. We identified Cdc14 as the phosphatase that dephosphorylates Hsp90 and disrupts its interaction with Mps1. This causes Mps1 degradation, thus providing a mechanism for its inactivation. Finally, Hsp90 phosphorylation sensitizes cells to its inhibitors and elevated Mps1 levels confer renal cell carcinoma selectivity to Hsp90-drugs. Mps1 expression level can potentially serve as a predictive indicator of tumor response to Hsp90 inhibitors.
PROFFERED PAPER:
The Tumor Suppressor Functions of PBRM1 in Kidney Cancer

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In clear cell renal cell carcinoma (ccRCC) the functional loss of von-Hippel Lindau tumor suppressor (VHL) is the founding event for tumorigenesis. In addition, it was found that 40% of ccRCC tumors harbored polybromo-1 (PBRM1) mutations, a component of a SWI/SNF chromatin-remodeling complex PBAF. Multiple lines of evidence suggest that PBRM1 is another essential tumor suppressor gene like VHL in sporadic ccRCC. It is not known how tumor-derived mutations disrupt its tumor suppressor functions and how to curb tumor growth of PBRM1-deficient cancer cells. We found that PBRM1 was a reader of lysine acetylated p53 and was required for the full transcriptional activity of wild-type p53. We also identified a 25aa sequence on PBRM1 that was necessary and sufficient for PBAF binding, and identified lysine acetylation on PBRM1 as a critical PBAF binding signal. Most tumor-derived mutations would destroy PBRM1’s binding to the PBAF complex. In an IHC study of ccRCC TMA provided by Fox Chase Cancer Center, we found that the loss of PBRM1 correlated with worse overall survival, while the losses of BRG1 or BRM, the enzymatic subunits of the PBAF complex, were associated with better overall survival. Consistent with this observation, BRM suppression in ccRCC cancer cells significantly retarded tumor growth elicited by PBRM1 loss in a xenograft model. Thus BRM might be a critical drug target for PBRM1-deficient cancer cells.
SESSION TITLE
Animal Models
Inhibition of miRNA-132/212 Suppresses VHL-Regulated Pathophysiological Angiogenesis in Zebrafish and Renal Cell Carcinoma

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Objective: Clear cell renal cell carcinoma (ccRCC) is the most common form of sporadic and inherited kidney cancer, both of which are highly associated with biallelic mutations in the von Hippel-Lindau (VHL) tumor suppressor gene and an activated PI3k-AKT pathway. Although upregulation of the miR-132/212 and disturbed VHL signaling have both been linked with pathogenic angiogenesis, no evidence of a possible connection between the two has yet been made.

Approach and Results: We show that miRNA132/212 levels are increased after loss of VHL in vivo and in vitro. We also show that PTEN levels are downregulated in models lacking or after endogenous VHL knockdown in WT cells, partially due to the action of miRNA132/212. Furthermore, in vivo, we show that blocking miRNA132/212 with antimiRs can significantly alleviate the excessive vascular branching phenotype in vhl−/− mutant zebrafish. Moreover, using endothelial cells and pericytes in a coculture system, we observed that VHL knockdown in HUVECs promotes endothelial cells’ neovascularization capacity, which can be inhibited by antimiR-132/212 treatment.

Conclusions: Taken together, our results demonstrate an important role for miRNA132/212 in angiogenesis induced by loss of VHL and suggest an interesting opportunity for pharmaceutical intervention using inhibitor of miR-132/212 to inhibit tumor growth for ccRCC patients.
Generation of a Mouse Model of Clear Cell Renal Cell Carcinoma

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Zurich Center for Integrative Human Physiology (ZIHP), University of Zurich, Switzerland

Patients with VHL disease frequently develop clear cell renal cell carcinomas (ccRCC) and sporadic ccRCC is the most frequent renal malignancy in the general population. A limitation of research into this disease has been the lack of an accurate mouse model of ccRCC that can be used to gain biological insights and to test new therapies. Based on combined renal epithelial cell-specific deletion of Vhl/Trp53/Rb1 we have recently generated the first mouse ccRCC model that reproduces the molecular, cellular and clinical features of the human disease. We are currently characterising these tumours at the genomic level. This autochthonous model has the significant advantage over xenograft-based tumour studies in that it will allow therapeutic intervention studies of tumours that form in the relevant physiological environment of the kidney and in the presence of a functioning immune system. We are employing a µCT-based longitudinal imaging approach to quantitatively monitor tumour volumes over time. We will genetically test the requirement for HIF1a and HIF2a in the formation of ccRCC by generating and analysing two different sets of quadruple mutant mice; Vhl/Trp53/Rb1/Hif1a and Vhl/Trp53/Rb1/Hif2a. We are also initiating studies that will test the efficacy of chemical inhibitors of HIF-1α and HIF-2α as well as immune checkpoint inhibitors in this mouse model of ccRCC.
Arginine — A Potent Refolder of Mutant pVHL — A Possible Therapeutic for the VHL Syndrome

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Previous work in our lab has shown that the pVHL tumor suppressor is a molten globule protein displaying metastability. By in-silico analysis of the structure and folding of the wild type pVHL we identified in its core an aromatic tetrahedron, essential for stabilizing the protein. This analysis predicted that various common oncogenic VHL mutations would disrupt the aromatic tetrahedron, leading to misfolding of pVHL. Using biophysical methods we confirmed the in-silico predictions for several VHL mutations, demonstrating that the mutant pVHL proteins have lower stability than the wild type, distort the core domain and as a result reduce the ability of the protein to bind its target HIF-1α and HIF-2α. In order to identify candidate chemical chaperones that would restore folding, and hopefully function of mutant pVHL, we employed a bacterial pVHL-EGFP based assay. This screen identified Arginine as a highly potent re-folder of pVHL and this was verified in vitro. This refolding also resulted in functional restoration, in vitro, of the mutant proteins to the level of the wild type. In preparation for testing Arginine for VHL in the context of the intact organism we used Drosophila and demonstrated evolutionary conservation of the conformation of pVHL and its fly homolog dVHL. We also found that transgenic pVHL expressed in flies lacking dVHL is capable of rescuing the mutant phenotype of the latter, indicating that the fruit fly is a useful model for studying the human pVHL [1, 2].

We have now extended these observations to two in vivo model systems. First, we generated a transgenic Drosophila model of the VHL syndrome by over-expressing oncogenic mutant versions of human pVHL in flies lacking endogenous dVHL. We found that ubiquitous expression of a pVHL transgene, carrying a mutation that causes misfolding of the protein, leads to lethality at the pupal stage. Feeding Arginine to these flies rescued the lethal phenotype. This was accompanied by apparent re-functionalization of pVHL evident by reduction of the level of the fly homolog of HIF-1α and by reduction in the level of ODD-GFP, a tagged fragment from HIF-1α through which pVHL normally binds HIF-1α.

Second, to begin translating these results to human setting we stably transfected human renal carcinoma cell (RCC10) lacking endogenous pVHL with any one of several mutant pVHL versions and explored the effect of Arginine on them. Our initial findings indicate that Arginine increases the cellular level of the soluble pVHL protein of certain misfolded pVHL mutants presumably reflecting elevation of their stability. This is accompanied by apparent restoration of pVHL function of targeting HIF-1α for degradation, evident by reduction in the level of HIF-1α compared to untreated cells. It remains to be shown that the HIF-regulated pathway in these cells is also restored.

Future work will aim at verifying whether the mechanism of action of Arginine in these in vivo models is as observed in vitro, and at characterizing the scope of pVHL mutations that respond to Arginine.

Supported by the VHL Alliance and the Israel Cancer Association

RCC: Molecular Pathways and Novel Therapies

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Over the last decade, several drugs were developed that are effective against clear-cell renal cell carcinoma (ccRCC). However, these drugs are not curative, and most patients with metastases succumb to the disease. ccRCC is characterized by inactivation of the VHL gene. VHL loss activates HIF-2, and constitutive HIF-2 activation is sufficient to restore tumorigenesis in VHL-reconstituted ccRCC cells. HIF-2 promotes cell survival, proliferation, pluripotency, and angiogenesis. However, HIF-2, a transcription factor, has been regarded as undruggable and most existing drugs target angiogenesis. Progress has been stalled by (i) lack of targets for drug development, and (ii) lack of knowledge about other pathways. We show that HIF-2 can be effectively inhibited by PT2399, a first-in-class antagonist developed by Peloton Therapeutics. PT2399 suppresses tumorigenesis in 56% (10/18) of ccRCC tumorgrafts (PDX) models studied. PT2399 has greater activity than sunitinib, is active in tumors progressing on sunitinib, and is better tolerated. Additional targets for drug intervention may be identified from the discovery of new pathways. We found that the BAP1 gene is mutated in 15% of ccRCC. Interestingly, BAP1 mutations tend to anticorrelate with mutations in a second gene, PBRM1, which is inactivated in 50% of ccRCC. Notably, whereas BAP1-deficient tumors tend to be of high grade, PBRM1-deficient tumors are of low grade. According to BAP1 and PBRM1 status ccRCCs can be divided into 4 molecular subtypes, which are associated with different outcomes in patients. Provocatively, the BAP1 and PBRM1 genes are located in the proximity of VHL on chromosome 3p, in a region that is deleted in the vast majority of ccRCC. These data support a model for ccRCC development where following an intragenic mutation in VHL and deletion of 3p, a mutation in the remaining allele of BAP1 vs. PBRM1 leads to tumors with different grade and aggressiveness. This model addresses a longstanding paradox, which is why germline mutations in VHL predispose to ccRCC in humans, but not in mice. Unexpectedly, in the mouse, Bap1 and Pbrm1 are on a different chromosome than Vhl. We asked whether simultaneous mutation of Vhl and Bap1 was sufficient to induce ccRCC, and found that mice with combined mutations developed ccRCC. Because BAP1 and PBRM1 are lost in tumors, understanding of the effector mechanisms downstream will be critical to the identification of new targets for drug development.
PROFFERED PAPER:  
Establishment of Novel Models of VHL Disease by Using Patient-Derived Induced Pluripotent Stem Cells

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Introduction and Objectives: One of the major obstacles of developing new treatment strategy for VHL disease is the lack of disease model. To resolve this problem, we have established VHL patient-derived induced pluripotent stem cells (VHL iPS) to recapitulate disease phenotypes both \textit{in vitro} and \textit{in vivo}.

Methods and Results: We have successfully established iPS cells from six patients with VHL disease. Those patients have developed RCC, hemangioblastoma, pheochromocytoma, and pancreatic neuroendocrine tumors. As for \textit{in vivo} experiments, we inoculated iPS cells into testes of NOD/SCID mice to examine if disease phenotypes of patients are recapitulated in xenograft tumors. We also focus on establishing hemangioblastoma cells \textit{in vitro} through the differentiation of VHL iPS to its tumor origin since there exist no cell lines for this disease. Our preliminary results from both \textit{in vivo} and \textit{in vitro} experiments indicated that VHL iPS possess the ability to differentiate all three germ layers, ectoderm, mesoderm, and endoderm.

Conclusions: Although wild type VHL allele needs to be disrupted by the CRISPR/CAS9 system, VHL iPS might be useful tools for the recapitulation and the drug discovery of VHL related tumors.
SESSION TITLE
Cancer Metabolism
KEYNOTE ADDRESS

Systems Approach to Metabolism

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Metabolism underlies essentially all biological processes, so it is not surprising that many human diseases involve alterations of metabolism at the cellular level. This principle applies not only to rare, Mendelian diseases caused by inherited mutations in metabolic enzymes, but also in complex diseases like cancer, in which extensive reprogramming of metabolism occurs during the acquisition of malignant features. Metabolic alterations in cancer are related in part to the fact that oncogenes and tumor suppressor genes regulate metabolic flux, such that mutations in these genes impose drastic alterations of metabolic activity in transformed cells. Mutations in the von Hippel-Lindau tumor suppressor provide an excellent example of this principle, as they activate a pseudo-hypoxic state characterized by major changes in glucose, amino acid and lipid metabolism. I will discuss fundamental principles of cancer metabolism, including the role of reprogrammed activities in supporting cellular fitness, promoting tumor growth and enabling some aspects of disease progression. I will also discuss the major analytical dimensions of modern research in cancer metabolism, including metabolomics, stable isotope tracing, and metabolic flux analysis, drawing on recent examples from the cancer metabolism literature to demonstrate the strengths and limitations of each approach. Opportunities to capitalize on altered metabolic states to improve cancer imaging and therapy will also be presented.
The Role of PGC-1 alpha in RCC

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The mechanism underlying lipid accumulation in clear cell renal cell carcinoma (ccRCC) remains largely unknown. Here we demonstrate that suppression of PGC-1α drives multiple metabolic hallmarks of ccRCC. We provide evidence that PGC-1α is suppressed by HIF-α/Dec1-dependent transcriptional repression. Suppression of PGC-1α inhibited mitochondrial function, promoted lipid accumulation, and increased expression of acyl-coA synthetases (ACSL). Inhibition of ACSLs in ccRCC cells abolished lipid droplets and suppressed cell growth. Conversely, expression of PGC-1α increased mitochondrial function, ROS production, oxidative damage, and sensitized ccRCC cells to cytotoxic therapy. PGC-1α expression suppressed ccRCC tumor growth in immune deficient mice. These studies demonstrate that suppression of PGC-1α recapitulates key metabolic phenotypes of ccRCC and provide a link between renal tumorigenesis, mitochondrial and lipid homeostasis.
Metabolic plasticity enables organisms to respond and adapt to changes in their environment. While the core components of most pathways of intermediary metabolism have long been described—consisting of ~3000 metabolic genes organized in pathways interconnected by 1000s of shared metabolites—, it remains poorly understood how the flow of these metabolites is rewired in different metabolic states. This question is particularly relevant in the context of tumors, as cancer cells are frequently starved for nutrients and exposed to toxic waste products due to a combination of increased nutrient consumption and dysfunctional vasculature. Exploring cancer metabolism also provides a system to address a more fundamental question of how metabolic pathways and extracellular cues cooperate to meet the energetic and biosynthetic needs of cells at different metabolic states of metabolic diseases. In the Birsoy lab, we combine a number of cutting-edge techniques—from the development of forward genetics tools (i.e. CRISPR-Cas9 technology) to metabolomics—to elucidate how cellular metabolism contributes to human disorders such as cancer and inborn errors of metabolism.
PROFFERED PAPER:
ER Stress in von Hippel-Lindau Tumor Suppressor Gene Mutant Kidney Cells and the Induction of Inflammatory Response

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Mutations in the tumor suppressor gene von Hippel-Lindau (VHL) are the major cause of clear-cell renal cell carcinoma (ccRCC) that may originate from chronic inflammation. However, the critical role of VHL mutation in development of ccRCC via inflammation remains poorly understood. Additionally, it has also been reported that VHL mutant cells show increased protein synthesis, which may result in chronic unfolded protein response (UPR). Prolonged endoplasmic reticulum (ER) stress caused by unresolved UPR is highly deleterious to renal cell function and is thereby implicated in the pathogenesis of kidney disease. Therefore, we hypothesize that unresolved ER stress can then induce inflammatory responses in the kidney containing VHL mutant cells. Our results show that ER stress markers including BiP and XBP1 increase significantly in VHL mutant kidney cells and VHL knockout mouse kidney, and IRE1 pathway is activated. Moreover, inflammation-related proteins, such as phospho-JNK (p-JNK) and nuclearly localized NFκB subunit p65, also increased significantly, both in vitro and in vivo. Interestingly, APY29, an inhibitor of IRE1α autophosphorylation, could reduce p-JNK expression and NFκB/p65 translocation in vitro and in vivo. Furthermore, the interaction between phospho-IRE1α and TRAF2 (the scaffold protein that links IRE1 to JNK and NFkB) is nearly abolished in APY29-treated VHL mutant kidney cells. APY 29 also decreases the chemotaxis ability of macrophage RAW cells toward VHL mutant kidney cells. Our results offer exciting new insights into the therapeutic potential of anti-inflammatory agents against ccRCC development.
SESSION TITLE
Immunology and
Tumor Microenvironment
KEYNOTE ADDRESS

Role of Co-Stimulatory Molecules and Immune Cell Checkpoints in Cancer Suppression

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The immune system is capable of recognizing tumors and eliminates many early malignant cells. However, tumors evolve to evade immune attack, and the tumor microenvironment is immunosuppressive. Immune responses are regulated by a number of immunological checkpoints that promote protective immunity and maintain tolerance. T cell coinhibitory pathways restrict the strength and duration of immune responses, thereby limiting immune-mediated tissue damage, controlling resolution of inflammation, and maintaining tolerance to prevent autoimmunity. Tumors exploit these coinhibitory pathways to evade immune eradication. Engagement of PD-1 by PD-L1 or PD-L2 results in tyrosine phosphorylation of the PD-1 cytoplasmic domain, recruitment of SHP-2 (a tyrosine phosphatase), and de-phosphorylation of TCR signalling molecules. This leads to attenuated TCR signalling and inhibition of T-cell immune functions, as shown by decreased production of inflammatory cytokines and growth factors, decreased expression of cell survival proteins, metabolic re-programming, and altered motility and duration of interaction with dendritic cells and target cells.

Blockade of the PD-1, PD-L1, and CTLA-4 checkpoints is proving to be an effective and durable cancer immunotherapy in a subset of patients in a broad variety of tumor types by allowing the patient to produce an effective anti-tumor immune response. Expression of PD-L1 by tumor cells or infiltrating immune cells correlates with responsiveness to PD-1 or PD-L1 blockade but is an imperfect biomarker since some patients who do not express PD-L1 still respond. Single agent administration of these agents achieves durable tumor regression in some patients, while combined anti-PD-1 plus anti-CTLA-4 therapy may enhance anti-tumor benefit. A broad range of other immune modulatory targets has been identified and are potential targets for synergizing with immune checkpoint blockade. Advances in understanding T cell coinhibitory pathways have stimulated a new era of immunotherapy with effective drugs to treat cancer.
Engineering T cells to Target Cancer

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Genetically-modified T cell immunotherapy has achieved unprecedented responses in hematologic B cell malignancies, and T cells modified with chimeric antigen receptors (CARs) have been granted Breakthrough Therapy designation by the FDA at multiple institutions. Despite having been developed in the academic setting, many T cell therapies are now entering an industry setting to be developed into commercial therapies to treat cancer. We will discuss the components and technologies used in making a T cell product, some of the factors considered to be important for efficacy, and recent results in hematologic malignancies. We will also discuss pre-clinical and clinical data on a CAR T cell developed for glioblastoma.
ER Stress and Inflammatory Response in VHL Inactivated Kidney Tissue

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Kidney cancer is a serious health care problem around the world. It is symptomless in the early stage and is refractory to most of the cancer treatments. The most prevalent form of the kidney cancer, renal cell carcinoma of the clear-cell type (ccRCC), is mainly caused by loss-of-function of the von Hippel-Lindau tumor suppressor gene (VHL). VHL protein is an E3 ubiquitin ligase. Its most prominent degradation target is the alpha subunit of the hypoxia-inducible factor (HIF). However, the etiology of ccRCC formation remains unclear. Recent animal model studies in our laboratory have indicated that the early event in ccRCC development is tissue inflammation and fibrosis. This interesting result provides important support for the concept that chronic inflammation is tumorigenic. It is also consistent with the epidemiological evidence linking inflammatory kidney disease and kidney cancer. In the current study, we explored the mechanism by which changes within the VHL mutant cells effect a systemic inflammatory response, and found that the metabolic abnormalities caused by VHL gene mutation lead to unresolved chronic endoplasmic reticular stress (ER stress), which in turn induces activation of JNK and NFkB, two major pro-inflammatory factors, via the activation of IRE1alpha scaffolding function. The wider implication of this tumorigenic process will be discussed.
PROFFERED PAPER:
Identification of Novel Oncogenic Pathways in Central Nervous System Hemangioblastomas

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Objectives: This work aims to identify new oncogenic pathways that drive hemangioblastoma development, in order to design innovative therapeutic strategies to tackle this disease. Moreover, we aim to establish new hemangioblastoma cell lines, which will nourish further research including the generation of HB animal models.

Scientific Background: Hemangioblastomas (HBs) are highly vascular tumors that develop in the Central Nervous System, mainly in the cerebellum, spinal cord and retina. These tumors are usually associated with Von-Hippel Lindau (VHL) disease but they can also appear sporadically. In contrast to their benign behavior, HBs often contribute to the morbidity and mortality of VHL patients due to the location of the tumor and the lack of efficient treatment.

The genomic landscape of VHL-associated HBs is underexplored. The tumors develop upon bi-allelic loss of VHL but it is unclear if there are additional oncogenic drivers that influence tumor development. Previous studies showed that other chromosomal abnormalities are present in these tumors, but the responsible underlying genes have not been identified so far.

In addition, HBs are highly heterogeneous tumors comprised of diverse cell sub-populations and the lack of cell lines and animal models that mimic the disease strongly impairs the research on HB biology and the search for new therapeutic strategies.

Methods: Next-generation whole exome sequencing was performed in VHL-associated hemangioblastoma tumors. Patient matched blood samples were used as germline controls. Copy number variations, tumor cell fractions and somatic point mutations were discovered using GISTIK 2.0, ABSOLUTE and MutSig methods. The identified mutations were independently confirmed by targeted DNA libraries sequenced using MiSeq. Functional studies were performed in vitro to test the activity of the identified mutations. In addition, hemangioblastoma cell lines were established from fresh tumors. The cell lines were characterized by FACS analysis, WB and single-cell RNA sequencing.
SESSION TITLE
Therapeutic Challenges
Targeting VHL Tumors with RTK Inhibitors

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**Background:** Surgical excision is the most common form of management for von Hippel Lindau (VHL) disease related lesions in 2016. Efforts are underway to better understand the underlying biology of VHL disease related manifestations and to develop effective systemic targeted therapy. Due to the relative availability of antiangiogenic therapy for other indications, several of these agents have been tested in patients with VHL disease.

**Methods:** A review of the available literature was performed and key studies were abstracted and presented.

**Results:** Antiangiogenic therapy was associated with relatively consistent reduction in the size of renal cell carcinomas in a small sample size of patients, and there are anecdotal reports of regression in central nervous system lesions. There are few if any reports of complete responses or major, sustained responses. Data suggest that angiogenic receptors differ between vessels in renal cell carcinomas and hemangioblastomas.

**Conclusions:** The current generation of targeted therapies provide modest improvement in renal lesions and inconsistent results in hemangioblastomas and other VHL related lesions. A better understanding of the vascular biology of hemangioblastomas and of renal cell carcinomas will foster the development of better treatments for these disease manifestations.
Microbiome Association with Cancer

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An association between oral health and pancreatic cancer risk has been observed in several large cohort studies. Previously, we measured antibodies to oral bacteria in prediagnosis blood samples from 405 pancreatic cancer cases and 416 matched controls, nested within the European Prospective Investigation into Cancer and Nutrition study. Individuals with high levels of antibodies against Porphyromonas gingivalis ATCC 53978, a pathogenic periodontal bacteria, had a twofold higher risk of pancreatic cancer than individuals with lower levels of these antibodies. To investigate this link and examine the potential for biomarker discovery, a total of 44 subjects, undergoing surgery for biliary and/or pancreatic disease, were recruited. We targeted and sequenced the V3-V4 hypervariable region of the bacterial 16S rRNA gene from 180 samples from multiple sites within the oral cavity: buccal mucosa on the right and left side, dental biofilm, saliva, and tongue dorsum. Bacterial communities were evaluated in the context of each patient’s diagnosis: neoplasm of the extrahepatic bile duct, neoplasm of the pancreas and chronic pancreatitis. Principal coordinate analysis revealed a clear partitioning by sample type, however, did not reveal any observable patterns with respect to BMI, gender, age and ICD10 diagnosis. Overall, the phyla Firmicutes, Actinobacteria, Bacteroidetes, Proteobacteria and Fusobacteria, dominate the bacterial assemblages of the oral cavity in accordance with previous findings. Interestingly, Porphyromonas gingivalis, a previously identified pancreatic cancer biomarker candidate, is present in 93% of our patient pool.
Early Data from VHLA’s Patient Databank

Ilene Sussman, Suzanne Nylander, VHL Patients and Researchers
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A significant outcome of the 10th International VHL Medical Symposium (2012, Houston, TX) was the VHL Alliance’s Research Council’s mandate to invest research dollars into a comprehensive and international natural history study to complement existing registries/databanks. Such a study would provide important information that could be used by VHL researchers world-wide including (indirect) assess to patients for future clinical trials and baseline data needed for regulatory approval of future therapies.

The Research Council strongly advised that the study be created and maintained by the VHL Alliance in order to avoid possible issues of proprietors by any given institutions and would allow for access of (de-identified) data to the international VHL research community.

Surveys were created by an international task force comprised of researchers, healthcare professionals, and patients. The Cancer in Our Genes International Patient (CGIP) Databank was launched in the spring of 2014 as a partnership of patients and researchers and a collaboration between VHLA and NORD (National Organization of Rare Disorders), the platform provider.

CGIP is designed as a longitudinal study to further the understanding of:

• VHL’s natural history independent of mutation variability possibly resulting from geographic barriers
• Impact of lifestyle factor on syndrome progression including tumor growth rate
• Long-term effect of experimental therapeutic approaches
• Commonalities and differences between VHL and other rare cancer syndromes such as BHD, HLRCC, and SDH

CGIP is a secure and confidential, IRB approved, online study collecting patient data internationally. Inclusion of data from minors (<18 years of age) allows for the capture of important information from an early age, through puberty, and into adulthood. Data curation is incorporated into the study design.

The value of CGIP was proven within a few months after its launch as intriguing information about oral health and VHL was identified. Further studies to fully understand the relationship and/or impact are needed. CGIP data is also providing insight into ways that patients can improve their general health.

As CGIP participation grows, the data and its value will increase. The VHL Alliance looks to the VHL community to maximize CGIP’s impact.
PROFFERED PAPER:
PT2385: HIF-2α Antagonist for the Treatment of VHL-Mutant ccRCC

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Objectives: Defect in the VHL protein is the major underlying cause of renal malignancy in patients with VHL disease and in patients with sporadic clear cell renal cell carcinoma (ccRCC). PT2385 is a first-in-class, highly selective, and potent small molecule HIF-2α antagonist. PT2385 has been evaluated in preclinical models of ccRCC to assess its potential as a therapeutic treatment for patients with sporadic ccRCC and for VHL disease patients with renal cell cancer.

Background: ccRCC occurs in ~70% of patients with VHL disease, and is the leading cause of death for these patients. ccRCC also occurs sporadically, and VHL has been found to be inactivated in ~90% of cases, as a result of mutation, chromosome loss, or promoter hypermethylation. pvHL inactivation results in stabilization of hypoxia-inducible factor-α (HIF-α) proteins leading to the expression of multiple genes essential for the initiation and progression of ccRCC. The HIF-α family of transcription factors consists of HIF-1α, HIF-2α and HIF-3α. The HIF-α proteins are subject to post-translational regulation in response to oxygen tension. Under normoxia the HIF-α proteins are hydroxylated on proline residues by the action of the dioxygenase HIF-specific prolyl-hydroxylases (PHDs). These modifications serve as recognition sites for pvHL, which, as part of an E3 ubiquitin-ligase complex, targets HIF-α proteins for proteasomal degradation. Preclinical studies have provided evidence to suggest that HIF-2α, and not HIF-1α, plays a pivotal oncogenic role in ccRCC. Utilizing medicinal chemistry supported by iterative structure-base drug design, we discovered a series of potent and selective HIF-2α antagonists, and PT2385 was selected for further evaluation of its anti-tumor activity in ccRCC.

Methods: PT2385 was evaluated for its ability to disrupt heterodimerization of HIF-2α with HIF-1β, and to inhibit expression of HIF-2α target genes. The anti-tumor activity of PT2385 was assessed using mouse xenografts that have VHL loss. The efficacy and safety of PT2385 in preclinical models was also compared to that of anti-VEGF/VEGFR agents currently used to treat ccRCC.

Results: PT2385 is a potent, selective and orally active small molecule HIF-2α antagonist that binds the PAS-B domain of HIF-2α and allosterically blocks its dimerization with HIF-1β. In VHL mutant ccRCC cell lines and xenograft tumors, PT2385 inhibits the expression of HIF-2α-dependent genes, including VEGF-A, PAI-1, and cyclin D1. Orally administered PT2385 causes dramatic tumor regression in VHL mutant ccRCC tumor-bearing mice. Interestingly, in xenograft models PT2385 inhibits tumor-derived human VEGFA with no effect on mouse VEGFA. Importantly, PT2385 has no adverse effect on cardiovascular performance, distinguishing HIF-2α antagonism from other anti-angiogenic mechanisms currently used to treat ccRCC. In mice, PT2385 suppressed HIF-2α gene product erythropoietin (EPO) both at the level of mRNA and circulating protein. In a related fashion, hemoglobin, hematocrit, red blood cells and reticulocytes were also reduced although the observed effects were modest and not dose limiting. In humans, EPO was reduced in a dosedependent manner in ccRCC patients after treatment with PT2385, demonstrating its potential utility as a pharmacodynamic biomarker in the clinic.

Conclusions: PT2385 represents a novel class of therapeutics for the treatment of renal cell cancer, with the potential for improved efficacy as well as tolerability compared to current agents that target the VEGF pathway. PT2385 is currently under evaluation in a Phase 1 study in patients afflicted with advanced ccRCC. Strong scientific rationale exists to support evaluation of PT2385 as a treatment for patients with VHL disease.
SESSION TITLE
Clinical Applications
KEYNOTE ADDRESS

Integrated Care for Cancer Patients

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One of the first challenges in optimizing care for VHL patients was setting up a network of clinical care centers offering multidisciplinary services and coordinated care to the patients. We have largely achieved that goal, and impact on patient perception of care has reflected positively on that development. Many centers represented in this session offer such coordinated care: expert centers that see sufficient patient numbers to maintain experience, often coupled with research. But the VHL patient is not just the sum of VHL-associated lesions; the next challenge is to offer integrated care. What are the holes that exist? Should a nutritionist be part of supportive care? Lifestyle management? Post- or pre-surgery physiotherapy? Family planning? Psycho-social support or stress management techniques? How can we recognize and address the gaps in patient experience to facilitate transition of clinical care centers to VHL Integrated Care Centers?
Posters, Abstracts
Liver Hemangioblastomas in Mice with Inactivation of Vhlh in Granulocytes

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We generated conditional Vhlh knock-out mice in which the murine Vhl gene, Vhlh, is inactivated in a subset of kidney tubules (HOXB7-Cre Vhlh KO mice). These mice develop hyperplasia and clear cell phenotype in the kidney epithelium, which is rescued by inactivation of HIF-1 alpha, but not by inactivation of HIF-2 alpha/Epas-1 (assessed in HOXB7-Cre Vhlh-HIF-1alpha or HIF-2alpha double knock-out mice; Pritchett, Bader et al., Oncogene 2015). Unexpectedly, HOXB7-Cre Vhlh KO mice also exhibit hemangiomas (vascular overgrowth) and extramedullary erythropoiesis in the liver. A similar phenotype has been previously described in mice with hepatocyte specific inactivation of Vhlh (PEPCK-Cre and Alb-Cre mice1,2. In the latter mouse models, erythrocytosis and hemangioma formation is accompanied by over-expression of Vegf-a and erythropoietin, and over-expression of these cytokines as well as hemangiomas and erythrocytosis, are rescued in Vhlh-HIF-2alpha double KO mice. In contrast, in our mouse model, Vegf-a is not significantly up-regulated. On the other hand, we observe up-regulation of Angiopoietin-2 (Angpt2), Pdgf-b and especially Placental growth factor (Pgf) (~100x up-regulation). HIF-2alpha inactivation (assessed in Vhlh-HIF-2alpha double KO mice) leads to normalization of Angpt2, Pdgfb, Pgf expression, as well as serum erythropoietin levels. However, although HIF-2alpha inactivation efficiently suppresses extramedullary erythropoiesis, it does not fully rescue hemangiomas.

Using the Rosa-LacZ reporter mice, we did not detect Cre activity in hepatocytes of HOXB7-Cre mice. Since Hoxb7 expression has been observed in murine neutrophils, we isolated granulocytes from hemangiomas of HOXB7-Cre KO mice by fluorescent activated cell sorting and detected Vhlh inactivation within this fraction using genomic PCR. By qPCR, we also observed up-regulation of Phd3, a well known HIF-2alpha responsive gene, within liver granulocytes, and by immunohistochemistry, we detected large numbers of placental growth factor positive neutrophils within the hemangiomas. Bone marrow transplant experiments corroborate that Vhlh inactivation in granulocytes contributes to hemangioma formation in this mouse model, but also show that Vhlh null granulocytes are not sufficient for induction of hemangiomas, indicating that another cell type is involved. Taken together, our data show that different cell types (hepatocyte vs granulocyte) and cytokines (Vegf-a vs Angpt2 and Pgf) underlie hemangioma formation in HOXB7-Cre Vhlh KO mice, compared to PEPCK-Cre mice. Similarly, VHL tumors may employ different pro-angiogenic cytokines depending on the cell type in which inactivation is taking place. Furthermore, of relevance for VHL patients, inactivation of VHL in granulocytes may result in a pro-angiogenic phenotype, and pro-angiogenic granulocytes, once recruited to tumors, could contribute to tumor angiogenesis.

1Rankin et al., 2005 Mol Cell Biol 25, 3163-72.
2Rankin et al. 2008 Oncogene 27, 5354-8.
3R. T. Sasmono et al., J Leukoc Biol 82, 111 (Jul, 2007)
Deregulated HIF-Responsive Demethylases Create a Dependence on the EZH1 Histone Methyltransferase in VHL Deficient Tumors

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Background and Objective: Inactivation of the pVHL tumor suppressor protein is the signature lesion associated with clear cell Renal Cell Carcinomas (ccRCCs), the most common form of kidney cancer in adults. pVHL functions as the substrate recognition subunit of a Cullin2-dependent Ubiquitin (Ub) ligase complex that targets the α subunit of the Hypoxia Inducible Factor (HIF) transcription factors for Ub-mediated proteolysis, in a manner that is exquisitely dependent on oxygen availability. VHL loss in ccRCCs, therefore, leads to constitutive HIF activation (unlinked from oxygen) and promotes a slew of biological programs that together support tumor growth. Consequently, drugs targeting elevated HIF activity, either directly or indirectly, have shown clinical promise. Unfortunately, these drugs only yield short term remissions, often with undesirable side-effects when combined with other cytotoxic agents. Identifying novel targets in VHL deficient tumors, therefore, could significantly improve ccRCC therapy.

Results and Conclusions: Genes encoding chromatin modifiers are frequently mutated in ccRCCs. Some chromatin modifiers, such as certain Jumonji (JmjC) family histone demethylases, are oxygen-dependent enzymes (dioxygenases) that are also HIF-targets. From an evolutionary perspective, induction by HIF possibly compensates for their lower activity in hypoxia. However, in VHL-/ ccRCC tumors HIF activation is unlinked from oxygen and in turn could lead to chronically deregulated levels and/or activity of these enzymes, thus causing global changes in histone modifications. Indeed, measuring such global changes shows that VHL-/ ccRCC tumors exhibit a distinct histone modification signature primarily defined by increased acetylation and hypomethylation of Lys27 on Histone H3 (H3K27), when compared to papillary and chromophobe renal cancers (which typically express wild type pVHL).

We reasoned that deregulated histone modifications at such a global scale could prove deleterious to the cell unless counteracted by other means. VHL-/ tumors possibly compensate for these deleterious changes via alterations in the levels and/or the activity of other chromatin modifiers. Using shRNA-mediated loss-of-function screens as a means to identify such compensatory changes, we identified several enzymes whose inactivation led to a fitness defect in VHL-/- cells. Systematically characterizing these candidates might yield therapeutically targetable dependencies.

Here, we show that the EZH1 H3K27 methyltransferase represents one such dependency in VHL deficient cells. EZH1 inactivation, both genetically and pharmacologically, preferentially impairs the growth of VHL-/ cells in vitro, and its pharmacological inhibition significantly impedes tumor growth in vivo. Mechanistically, in VHL deficient cells, EZH1 likely counteracts HIF-mediated induction of the reciprocal H3K27 histone demethylase, KDM6B. Together, these results implicate EZH1 as a novel target in kidney cancer and provide the rationale for the development of pharmacological agents that specifically target EZH1 activity. Because the canonical role of EZH1 (H3K27 methylation) is associated with transcriptional silencing, it is likely that EZH1 silences key tumor suppressor networks in VHL-/ ccRCCs. Identification of the critical targets of EZH1 in these cells might therefore uncover key tumor suppressive pathways and inform us of novel ways to target renal tumors.
von Hippel-Lindau (VHL) Clinical Care Center (CCC) in Buenos Aires, Argentina

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Background: VHL disease is a rare autosomal dominant disorder characterized by the development of cysts, benign and malignant tumors in different organs that may lead to multi-organic affectation and health impairment. Multidisciplinary approach in specialized units with trained staff is mandatory. The Hospital Italiano de Buenos Aires (HIBA) was designated as VHL CCC in December 2014.

Objectives: Introduce the creation of the VHL Unit, its organization, aims and working procedures, and share our experience.

Methods: HIBA is a first level reference and university hospital equipped with the highest technological and scientific resources to provide complete medical care and research for VHL disease. The main goal of the VHL unit is to offer specialized, comprehensive and coordinated assistance, accurate diagnosis and genetic counseling. A clinician coordinates the appointments with the different specialties. Patients are followed by structured online health record based on an electronic health chart.

The unit works in partnership with the Hospital de Niños Ricardo Gutierrez, a children’s referral hospital with an extensive experience in VHL disease. Patients are referred there for genetic testing. Regular meetings among professionals involved are performed on a monthly basis. Our website provides updated information for patients and professionals.

Results: Fifteen patients were followed, ranging from 25 to 62 years old. Twelve female (80%). The most frequent tumors found were: central nervous system hemangioblastomas (73.3%), pancreatic cysts (60%), retinal angiomas (53.3%), renal cell carcinoma (40%) and pheochromocytoma (20%). Four patients (26.6%) had significant complications: unilateral amaurosis (3), bilateral hearing loss with cochlear implant (1), adrenal insufficiency (1) and end stage renal disease (2). Twenty percent of the patients presented adjustment disorders with depressed mood. Genetic testing was performed in 10 patient (8 confirmed VHL).

Conclusions: The VHL Unit provides a multidisciplinary approach for patients and their families, offering adequate and integral assistance and promoting knowledge and scientific advances in the area. Future plans include informative meetings for patients and research projects to better understand the nature of the disease.
Evidence for Genetic Anticipation in von Hippel-Lindau Syndrome

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Objective/hypothesis: This study will assess if genetic anticipation exists in VHL through comparison of the age of onset (AOO) over multiple generations in familial cases. The results will aid in a review of the current surveillance recommendations for VHL patients.

Background: Von Hippel-Lindau (VHL) is a rare hereditary tumour predisposition syndrome inherited in an autosomal dominant pattern. Patients are susceptible to benign and malignant tumour growth in various visceral organs and the central nervous system. Management of VHL involves frequent, lifelong surveillance for tumour growth and treatment of symptoms when they arise.

Methods: A retrospective review of all VHL families (n=27) at Sick-Kids was undertaken. Only patients with familial VHL and sufficient information to confirm a diagnosis and AOO for a minimum of two generations were included in the study (n=14 unrelated families, n=76 patients). A paired Wilcoxon Signed Rank test was used to determine if the AOO varied significantly between generations (n=60 parent-child pairs). AOO was defined as either: the age at which a VHL tumour was diagnosed, or the age at death if confirmed to have VHL.

Results: There was a significant difference in the AOO between the children and parents (p<0.001) and parents and grandparents (p=0.009).

Conclusions: Genetic anticipation is apparent in VHL as the AOO was significantly different between generations concluding that subsequent generations have an earlier age at which symptoms first arise. These results indicate a need to review current surveillance protocol recommendations for children with familial VHL.
Clinical Management and Evolution of Patients with von Hippel-Lindau Disease and Pancreatic Neuroendocrine Tumors

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Introduction: Pancreatic neuroendocrine tumors (PNET) are potentially life-threatening lesions in von Hippel-Lindau (VHL) patients and often pose difficult questions of management. We aimed to describe the clinical management and evolution of VHL-related PNET in a large multicentre cohort.

Patients and methods: The data of all patients with PNETs included the French VHL cohort (n=780) between 1991 and 2015 were retrospectively reviewed with focus on diagnostic modalities, management and evolution features. Patients with insufficient data were excluded of the study.

Results: The diagnosis of PNET was assessed in 68 VHL patients (8.7%). Among the 45 patients in whom accurate data were available, 14 (31%) were male and the median age at PNET diagnosis was 36.4 years (Interquartile range [IQR], 27.8-45.4). Pancreatic cysts and serous cystadenomas coexisted with PNET in 28 patients (62%) and 18 patients (40%), respectively. The most prevalent VHL gene disorders were deletions, or exon 1, exon 2 or exon 3 mutations in 9 (20%), 10 (22%), 4 (9%) or 18 (40%) patients, respectively. PNET were diagnosed by systematic screening during follow-up in 38 (84%) patients, or because of symptoms in 7 patients. The median number of PNET per patient was 2 (IQR 1-5), and the size of the largest lesion was 26 mm (IQR 22-35). Metastatic lymph nodes were present in 16 patients (36%) and median proliferation index Ki67 was 4.5% (IQR 1.5%-7.3%). Among the 31 patients (69%) who underwent a parenchymasparing surgical resection, 21 patients had an upfront surgery and 10 patients were operated on during the observation period. The indication for surgery was the size of PNET in all of them but three who had pain and/or acute pancreatitis. A tumor progression of small PNET left in place during initial surgery occurred in 3/31 patients. Eight patients (18%) in whom only follow-up was proposed had no tumor progression. Distant metastases were present at diagnosis in five patients (11%) or developed after a median follow-up of 9.2 years in seven patients (16%). Metastatic PNET were treated with cytotoxic chemotherapy (7/12), targeted therapy (6/12, mainly sunitinib), somatostatin analogs (3/12) and/or intra-arterial hepatic chemo-embolization (2/12). After a median follow-up of 10.2 years (IQR 5.3-14), eight patients (17.8%) have died, due to the course of PNET in four of them.

Conclusion: Despite the growth of VHL-related PNET is slow, the risk factors of a metastatic course are not entirely known thus a life-long follow-up remains necessary. Advanced PNET require decisions through medical-surgical multidisciplinary meetings with experts in both pancreatic and VHL diseases.
Role of pVHL_{172} Isoform in Tumorigenesis


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Objectives: The human VHL gene encodes two transcripts (mRNA1 and mRNA2) and three different protein isoforms (pVHLs). The pVHL_{213} and pVHL_{160} exhibit a tumor suppressor function in renal cancer cells while the effect of pVHL_{172} has never been investigated for this function. We decipher the function of the pVHL_{172} isoform in VHL syndrome patients and more specifically in clear cell renal carcinoma (ccRCC) tumorigenesis.

Introduction: The human pVHL which acts as a tumor suppressor is found mutated in ~80% of sporadic ccRCC. The mRNA variant 1 encodes the full length pVHL_{213} and a shorter pVHL_{160} version produced by using an internal translation start site. The mRNA variant 2 encodes pVHL_{172} in which exon 2 is alternatively spliced. We recently showed that these isoforms are co-expressed in human renal cells and ccRCC tissues (Chesnel et al., 2015). Moreover, the ratio of the two mRNA variants is modified in several ccRCC tumors, sometimes in favor of the second mRNA variant (Taylor et al., 2012).

Methods: We generated stable cell lines which expressed the pVHL_{213} or pVHL_{172} in 786-O cells. We characterized phenotypically and biochemically the cells. We then generated tumors in mice with cells expressing pVHL_{213}, pVHL_{172} or no pVHL and characterized their growth and phenotype.

Results: The pVHL_{172} expressed in cells induces alteration of cell proliferation but not motility or clonogenicity. The ability of 786-O pVHL_{172} cells to induce tumor formation differed from what we observed with the 786-O or 768-O pVHL_{213} cells.

Conclusion: Our work suggests that pVHL_{172} alters progression of tumors generated from 786-O cells.
Laser Photocoagulation for Retinal Capillary Hemangioma in von Hippel-Lindau Disease

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Objectives: Describe the efficacy and tolerance of laser photocoagulation for retinal capillary hemangiomas (RCH) in von Hippel Lindau (VHL) disease and to define the conditions in which this treatment is clinically optimal.

Introduction/Background: The treatment of RCH is based on ablative therapy unless RCH are localized close to the optic disc or the macula. Selection of a treatment modality for RCH may depend not only on the location but also on the size of the RCH. Small lesions can be simply observed or photocoagulated with laser. Bigger lesions can benefit from transconjunctivo-scleral cryotherapy.

Patients: The charts of 71 patients who had presented a RCH treated by laser photocoagulation in one or both eyes were retrospectively reviewed. The aim of the treatment was to destroy the RCH by long-duration (0.5-1 s) green laser burns. The RCH obliteration was monitored and the evolution of the retinal exudation around the RCH was assessed.

Main Outcome Measures were the size of the RCH at diagnosis, the presence of retinal exudation, pre and post treatment visual acuity. Outcome assessment included regression of the tumor, and complication of the treatment.

Results: 304 RCH in in 95 eyes of 71 patients were identified. Mean length of follow-up was 4,5 years (0,1 to 17,5). Two hundred and thirty four RCH (77%), were coagulated in a single laser application. The median size of these RCH was 0.25 disc diameter (DD) (0.25 to 1.5). They were not associated with any retinal exudation before treatment. No complication occurred after laser or during the follow up. Mean VA remain stable during the follow up (initial 0.9, final 0.8). Seventy RCH (23%) needed more than one laser application to be coagulated. The median size of these RCH was 1 DD (0.25 to 3). Mean VA remained stable during the follow up (initial 0.9, final 0.8). Of these 70 RCH, 29 (10% of all the cohort) needed additional laser session during the first 48 hours. Their median size was 1.5 (0.5 to 3). VA also remained stable during the follow up (0,8 to 0,7). Retinal exudation was associated before treatment with 15 RCH of which the median

Identification of Novel size was 2 DD (1,5 to 2,5). In these cases exudation increased transiently in 6 cases after the first laser session but completely resolved in a few days after prompt laser retreatment. In 5 other cases without initial Retinal exudation, serous retinal detachment nevertheless occurred after the first laser session. The median size of these RCH was 1,5 DD (1,5 to 3). The exudation completely resolved in 3 cases after prompt laser retreatment, but in 2 other, a rapid worsening of a vitreoretinal traction on the RCH resulted in a tractional and rhegmatogenous retinal detachment, which needed vitreoretinal surgery.

Conclusion: Laser therapy allowed us to handle large RCHs (up to 3 DD), even when there was a retinal exudation. Those cases require close patient monitoring with long and intense laser impacts. Associated exudation is not a contraindication to laser photocoagulation.
von Hippel-Lindau (VHL) Development in Children and Adolescents

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Objectives: We aimed to describe how often and in what way VHL clinically affects children and adolescents, focusing on age at diagnosis, age at first manifestation and the most frequent manifestations.

Introduction: Previous studies of VHL development have mainly focused on adults. Knowledge of paediatric VHL development and manifestation burden is limited, and current surveillance guidelines are based on best clinical judgement. Increased knowledge of VHL progression in children and adolescents could improve childhood surveillance, and more accurate information about VHL development during childhood could help decrease worry and distress in VHL families.

Methods: We included almost 90% (84 of 96) of all known VHL mutation carriers in Denmark. Information about all manifestations diagnosed before the age of 18, was collected through participant interviews and validated by medical records. We evaluated disease progression in childhood based on 84 patients who had been diagnosed with VHL before the age of 18; 36 Danish patients as well as all systematically reported VHL-patients under the age of 18 from the international literature (N=48).

Results: Forty-three percent of the Danish VHL-patients (36 of 84) had been diagnosed with VHL (either clinically or molecularly) and initiated surveillance before the age of 18, and more than 80% of the manifestations (62 of 77) were found because of surveillance. Of the 84 Danish and international patients diagnosed with VHL in childhood or adolescence, 70% (59 of 84) had developed manifestations before 18 years. The median age at first manifestation was 12.5 years (range: 5-17 years). Thirty percent of both the Danish and international patients (25 of 84) had developed more than one type of manifestation; the most frequent types were retinal (43%) and CNS (29%) hemangioblastomas.

Conclusions: Surveillance ophthalmoscopies are essential to minimize the risk of blindness and regular MRIs of the CNS should be considered to be initiated in childhood.
Survival and Causes of Death in von Hippel-Lindau (VHL) Patients

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Objectives: How has the survival of VHL mutation carriers changed over time and how often do VHL patients die from VHL-related causes?

Background: Historically, the survival of VHL patients has been poorer than that of the general population. Implementation of surveillance during recent decades is believed to have improved survival markedly, but evidence is missing. We uniquely demonstrate how survival has improved over the last 115 years in a systematic cohort study including all known Danish VHL mutation carriers.

Methods: We included 143 VHL patients born between 1900 and 2010 (67 deceased and 76 living) from 34 unrelated families with disease causing VHL mutations. We collected their dates of birth and death, as well as their causes of death, through the national Danish Death Register, death certificates, autopsy reports, and medical records. The survival of patients born in different periods was estimated using Kaplan Meier curves. Then survival times were compared in a Cox-regression model which allowed correlated life spans within families. We will also present comparisons of survival times in VHL patients and their unaffected relatives as well as the general Danish population.

Results: For patients born between 1900 and 1955, Kaplan Meier curves showed survival probabilities of 95%, 71%, and 46% at ages 20, 40, and 50 years, respectively. For patients born after 1955, the survival probabilities for the same ages were 100%, 95%, and 86% (similar to the general Danish population). The Cox model showed a highly significant decline in death rates of 79%, (95%CI 54%-90%, p<0.001). Overall, 84% (47 of 56) of deaths among VHL patients were caused by VHL-related illness, most commonly CNS hemangioblastomas (45%, 21 of 47) and metastasizing renal cell carcinoma (40%, 19 of 47).

Conclusions: Survival of VHL patients has improved markedly for patients born after 1955, the majority of whom have attended surveillance during their adult lives. Despite a survival comparable to the general population, VHL-related disease is the cause of death in most patients, also in those born after 1955.
Cancer in our Genes International (CGIP) Databank: A Part of NORD’s RareHistory Study

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The NORD RareHistory platform is a Web-based platform for natural history studies. Its primary goals are security of data, customizability, full data versioning, and respondent ease of use and engagement. The platform is managed by NORD and employs, among other technologies, C#, ASP.NET MVC, WebApi, AngularJS, Entity Framework, and Azure SQL and hosting. Data is secured with a defense-in-depth strategy: all pages are served securely over HTTPS; sensitive data is encrypted at-rest with AES (Advanced Encryption Standard), a form of encryption the US government has deemed appropriate for top-secret information; and each study has its own unique, computer-generated encryption key, stored outside of the database, so that even in the event of a database breach sensitive data cannot be unscrambled. The forward-looking design of the RareHistory platform allows respondents to register themselves and begin answering surveys for themselves and others and provides tools for those running the study to analyze data and to export it to share with researchers (with the ability to strip out any potentially identifying information). Furthermore, its full customizability means that it can continue to adapt as the needs of the studies hosted in it change.
Endolymphatique Sac Tumor: Characterization and Management in von Hippel-Lindau Disease

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Objective: Report the characterization of the endolymphatic sac tumors (ELST) in von Hippel-Lindau (VHL) disease and analyze the difference with sporadic cases.

Patients and Methods: Fourteen cases of ELST, occurring since 1998, were reviewed. We analyzed the initial symptoms, characteristics of the tumor, treatment, sequelae and follow-up for each group.

Results: Endolymphatic sac tumors (ELSTs) are, with a prevalence of 3.6 to 16%, a component of von Hippel–Lindau (VHL) disease. The prevalence of VHL germline mutations in apparently sporadic ELSTs was 39%. ELST was the initial manifestation in 32% of patients with VHL-ELST. The mean age at the time of the first surgery was 26 years [12-41] for the ELST associated with VHL disease. Most of the patients presented with a unilateral tumor. The initial symptoms were hearing loss, tinnitus and/or vertigo. Preoperative arteriography should be performed for all patients, but embolization is frequently difficult to perform. The size of the tumor was significantly larger in the sporadic cases (31.7 mm) than in cases of VHL disease (19.3 mm). Thus, the surgical approach was more extensive in the sporadic cases. The surgeons found two types of tumors. Cystic tumors with massive bleeding invading the surrounding structures (dura mater or jugular bulb) were more common in the sporadic cases. Fibrous tumors that infiltrate bone and have moderate bleeding were more common in the cases associated with VHL disease. Small lesions have a very low tumor growth. Four recurrences occurred during the fourteen years of follow-up. Four facial palsies and eight cases of profound deafness were encountered postoperatively.

Conclusion: Sporadic tumors are more aggressive than those associated with VHL disease. Complete surgical resection should be the goal of treatment. Preoperative angiography with embolization is mandatory. In some cases, embolization could be impossible, and pre- or postoperative radiotherapy should be discussed.
Vhl Inactivation in the Zebrafish Pronephros Models Early Stage Human Clear Cell Renal Cell Carcinoma

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Inactivation of the Von Hippel-Lindau (VHL) tumor suppressor gene is the first molecular lesion seen in over 90% of sporadic clear cell renal cell carcinoma (ccRCC) cases. Patients with VHL disease have a germline mutation in the VHL gene leading to the development of ccRCC among other tumors. Inactivation of VHL results in the stabilization of Hypoxia Inducible Factors 1a and 2a (HIF1a/HIF2a) leading to up-regulation of HIF target genes involved in angiogenesis, cell proliferation, and erythropoiesis. “Clear cell” tumors are classified by the presence of large, proliferating cells with “clear” cytoplasm and a reduction in cilia. Zebrafish with a homozygous inactivating mutation in both copies of the vhl gene (vhl−/−) recapitulate several aspects of the human disease, including angiogenesis in the brain and retina resembling hemangio-blastomas, and erythrocytosis. Here we have characterized the epithelial abnormalities present in the pronephros of the vhl−/− zebrafish larvae as first step in building a model of ccRCC in zebrafish. Our data show that the vhl−/− pronephros exhibits an enlarged tubule diameter with a disorganized lumen and cilia, aberrant cellular proliferation, and abundant cytoplasmic lipid droplets. This phenotype is reminiscent of human “clear cell” histology. We believe we have characterized for the first time a model of early stage ccRCC in zebrafish. These abnormalities are partially rescued by treatment with a small molecule HIF2a inhibitor indicating this phenotype is at least in part, HIF2a dependent. The partial attenuation of the vhl−/− pronephric abnormalities highlights the potential use of the zebrafish model in drug discovery for the treatment of VHL disease and ccRCC.
Surgical Resection of Medulla Oblongata Hemangioblastomas: Outcome and Complications

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Objectives: The purpose of this study was to analyze the surgical outcome and complications of a single center series of medulla oblongata (MO) hemangioblastomas.

Methods: We retrospectively reviewed the medical charts of all medulla oblongata hemangioblastomas operated in our institution between 1996 and 2015. All patients had a pre- and post-operative MRI and a minimum follow-up of 6 months. Patients were scored according to the Karnofsky Performance Scale (KPS) and McCormick scale at the moment of admission, discharge and the last follow-up.

Results: Thirty one surgical procedures were performed on 27 patients (16 females and 11 males). The mean age was 33 years and 93% of patients had von Hippel-Lindau (VHL) disease. Three patients experienced very complicated post-operative courses, with one case ending in the death of the patient. Two patients required tracheostomy. According to McCormik’s classification, 7 (23%) of the 31 operations resulted in aggravation and 23 (74%) in no change. Considering the seven patients with aggravation at discharge: 4 patients (60%) returned to their preoperative status, 1 patients (14%) improved but remained below his preoperative McCormick grade, 2 patients (29%) did not improve. At last follow-up, KPS was ameliorated in 53%, stable in 40% and worsened in 7% of cases.

Conclusion: Surgery of medulla oblongata hemangioblastomas is a challenging procedure characterized by an acceptable morbidity. Transient morbidity is not negligible even if the long-term outcome is in most cases favorable. A compromised neurological condition seems to be the best predictor of unfavorable outcome.
Establishment of a Rare Disease Patient Registry

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Objectives: The Cancer in our Genes International (CGIP) Databank is a partnership between patients and researchers, and was developed at the recommendation of the VHLA Research Council. VHL, BHD, HLRCC, SDH and other related genetic risks for cancer are different from many other conditions that cause tumors to grow. In order to preserve the functions of the affected organs, tumors are watched over time and only removed when necessary. This gives researchers a unique opportunity to understand factors affecting tumor development and growth rate. The international reach of this project also removes possible bias due to specific mutations from specific VHL families living in certain geographic areas, and allows analysis of treatment methods across regions.

Introduction/Background: CGIP launched in March 2014. Announcements were made to VHL Alliance constituents in a variety of written and online communications, and on social media. The VHL Clinical Care Centers were also informed of the launch of this important research initiative. Printed brochures describing the Databank were mailed to VHL Alliance constituents, distributed to patients at the VHL Clinical Care Centers, and included in meeting materials at the VHLA Annual Meeting. Individual participants also wrote about their experiences and reasons for participation.

One year after launching CGIP, VHLA reached out to patients who had not yet participated in order to learn their reasons. We were surprised to learn that the overwhelming reason was that they had never heard of the Databank. Following this, we increased individual outreach and held online training sessions to help interested patients experience using the Databank website and obtain help in registering. Local VHLA chapters also discussed the Databank at in-person meetings.

Methods: The CGIP Databank consists of data entered into online questionnaires either by the patients themselves, or by a family member with the patient’s consent. The data includes important lifestyle information on nutrition, exercise, and stress management, medications (prescription and over-the-counter), other medical conditions, and mental outlook. Questions were developed using an international task force that included both researchers and patients.

Patient-entered data is curated by the Databank Coordinator at the VHL Alliance with the exception of images and scans which will be read by a radiologist to establish consistent measures of tumor size and location. A major challenge is obtaining copies of patients’ records and scans as well as completion of surveys a second time to obtain longitudinal data. The VHL Alliance is starting a new outreach effort in 2016, using volunteers who have completed their own surveys and submitted their medical records to encourage others to do the same.

Results: As of March 2016, 579 have registered in the Databank, and 389 have created Participants and answered surveys. As we make progress and increase participation, we expect that the number of participants will outnumber the registrants. Each registrant is encouraged to create participants for affected children, elderly relatives, or those without access to a computer, and deceased relatives who had one of the diseases.

Implications: The large number of participants from around the world is already allowing us to take a look at data which may answer some common patient concerns/questions such as whether specific diets or regular exercise can reduce or accelerate disease progression. The Databank thus becomes a valuable starting point for clinical researchers interested in studying subgroups of VHL patients. The Databank is significantly shortening the time to learn about new aspects of VHL. Without the Databank, it took years to determine that Endolymphatic Sac Tumors (ELSTs) were a manifestation of VHL. The Databank is expected to reveal other information that may allow early intervention, medical or lifestyle, that will improve life for those with VHL and related rare diseases.
RSUME is Co-Expressed with VHL and Inhibits its Function

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Introduction: Inactivating mutations of pVHL provide a permissive setting for HIFs-α deregulation and development of high vascularized tumors, such as hemangioblastomas, renal clear-cell carcinomas (RCC) and pheochromocytomas.

RSUME (RWD-domain-containing sumoylation enhancer) is highly expressed in tissues sensitive to VHL disease. Data from RCC samples (Cancer Genome Atlas Research Network) showed that 4% of the tumors expressed high levels of RSUME and those patients exhibited a decrease in the survival rate. In hypoxia, RSUME increases HIF-1 stability and activity.

Objectives: Our aim were to investigate: a) the action of RSUME on pVHL-dependent HIFs-α stabilization, b) the mechanism by which RSUME acts on pVHL, and c) RSUME impact on pVHL-related tumor progression.

Methods: RSUME expression in human tumor samples and RCC-786-O cells was evaluated by RT-PCR, Western Blot (WB) and Immunofluorescence. HIFα stability was evaluated by WB and HIF stability reporter (ODD-LUC). VHL/RSUME interaction was evaluated by pull-down of recombinant proteins or by co-immunoprecipitation of COS-7 and RCC cell extracts. VHL sumoylation studies were performed by affinity purification of sumoylated proteins from transfected cells expressing His-SUMO-2 and by in vitro sumoylation assay.
Advancing Biomedical Research through Programmatic Initiatives

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NDRI is the leading national provider of human tissues for research into common and rare diseases. NDRI empowers research in many areas, including diabetes, HIV, and rare diseases such as Cystic Fibrosis, amyotrophic lateral sclerosis (ALS), lymphangioleiomyomatosis (LAM), and von Hippel-Lindau disease. NDRI establishes partnerships with Rare Disease Voluntary Health Organizations (VHO’s) by registering active researchers and facilitating donation for research. It is paramount for program success that an active research demand exists. NDRI works with partnering VHO’s and registered researchers to develop specific biospecimen protocols and recover tissues utilizing an established national network of recovery personnel. These individuals are trained and qualified pathologists, pathologist assistants, certified tissue bank specialists and trained tissue recovery staff located throughout the USA. NDRI’s Private Donor Program collaborates with the VHO’s to recover and distribute tissues from patients who participate in such programs and who have provided consent for the recovery of tissues and organs for research. For patients and families, the opportunity to donate for research that may lead to new treatments or cures offers comfort and hope.
Autophagy and Lysosomal Rupture to Target VHL-Inactivated Renal Cell Carcinoma

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Introduction: Kidney cancer is the eighth most common diagnosed cancer in the country. Despite the growth in therapeutic options, complete and durable response in metastatic RCC remains rare and 5-year survival rates are still low (10-20%). Tumor heterogeneity is particularly challenging in Renal Cell Carcinoma due to the variability in mutations in these tumors. One approach to develop therapeutic option is to target mutations present early in tumor development. It is estimated that up to 85% of RCC are due to mutations that inactivate the von Hippel-Lindau (VHL) tumor suppressor gene. We have previously shown the feasibility to target the loss of VHL by identifying a small molecule, STF-62247, that specifically eliminates renal cancer cells through autophagy.

Objective: This study aims to further understand the mechanistic of this small molecule by investigating the autophagy process.

Methods: We used quantitative proteomics and ingenuity pathway analysis to identify pathways involved in response to STF-62247. In addition, western blot analysis, confocal fluorescence microscopy, flow cytometry and clonogenic assays were used to characterize the autophagy process.

Results: We identified 755 proteins that were differently expressed in response to STF-62247. Ingenuity Pathway Analysis and DAVID identified autophagy as an important mediated-pathway. Inhibition of mTOR pathway through AMPK, Akt and amino acids is observed leading to decrease in protein synthesis. By characterizing the autophagy pathway, we found an accumulation of autophagic vacuoles over time in STF-62247-treated VHL-deficient cells, which are not degraded by lysosomes. Furthermore, STF-62247 induced lysosomal rupture that lead VHL-deficient cell to death. Finally, we showed that STF-62247 is localized to lysosomes and that VHL-deficient RCC can be sensitized by lysosomal disrupting agents.

Conclusion: This work revealed a role for VHL in autophagy lysosomal degradation that could be exploited in a therapeutic approach based on synthetic lethality to treat RCC with inactivation for VHL.

Funding. This study is funded by CIHR from Dr. Turcotte. She was recipient of a KRESSENT New Investigator (2012-2015)
Evolution of Renal Clear Cell Carcinogenesis

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VHL disease is a classic tumor suppressor gene syndrome characterized by development of specific types of tumors in selective organs with nervous system and kidney being most consistently affected. Detailed studies on human spinal cord and cerebellum have previously revealed earliest stages of CNS tumorigenesis and the morphologic sequence resulting in development of frank tumors.

To elucidate earliest stages of renal clear cell carcinoma, we performed a similar approach in kidney tissues of four VHL patients and three sporadic control cases. From all cases, blocks of interest were procured, followed by serial sectioning and 3dimensional reconstruction of potential precursor lesions. The results reveal an abundance of foci with aberrant mesonephric clear cell proliferations that initially develop along the tubular lining, but have the potential to aggregate within individual tubules. This stage is followed by microscopic invasive clear cell aggregations which represent tumor precursor structures.

This study presents evidence for a consistent morphologic sequence for renal clear cell carcinogenesis. Molecular analysis of early steps within this sequence will allow for identification of earliest genetic and proteomic changes in the future. Therapeutic targeting of earliest changes may allow to develop preventive strategies for renal cancer development for VHL patients.
Metabolic Contributions from the Pentose Phosphate Pathway in VHL(-) Clear Cell Renal Carcinoma

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Objectives: To determine contributions of the oxidative and non-oxidative branches of the pentose phosphate pathway (PPP) to the nucleotide synthesis in renal clear cell carcinoma.

Background/introduction: PPP is a classic anabolic pathway branching from glycolysis after glucose phosphorylation by hexokinase to form Glucose-6P. Cancer cells extensively utilize PPP for the generation of nucleotides for nucleic acid synthesis and for the generation of NADPH consumed during lipid synthesis and reduction of glutathione, supporting growth and proliferation of cancer cells. Activation of PPP is recognized as an important oncogenic metabolic pathway in ccRCC based on the high correlation of essential PPP enzymes, G6PD (Glucose-6-phosphate dehydrogenase), TKT (transketolase), and TALDO1 (transaldolase), with poor survival, as determined by TCGA. The oxidative branch is one of the main, if not the most important source of NADPH in cancer cells. However, it is believed that in cancer cells the majority of nucleic acid ribose results from the activity of the non-oxidative branch. Here, we use mass spectrometry coupled with liquid chromatography (LC-MS) for the direct detection of PPP intermediates to determine contributions of each branch of PPP to the generation of PRPP and nucleotides.

Methods: We have generated 786-O VHL(-) cell lines with knockdowns of the rate limiting enzymes in the oxidative and non-oxidative branch, G6PD and TKT respectively. Control cells expressed scramble shRNA. Cells were plated DMEM/F12 medium containing 5.5 mM glucose and 1% dialyzed FCS. Glucose consumption was monitored until the medium achieved concentrations of 2 mM and then cells were fed glucose to the original concentration. Cells were collected at time 0, and 15 and 90 minutes after addition of glucose. Supernatants containing metabolites were analyzed LC-MS. Mass spec data were collected in negative ionization modes, using ion paired reverse phase liquid chromatography with a Hydro-RP™ 2.5-μm C18 stationary phase column from Phenomenex®. Mobile phase A consisted of 5 mM diamyl ammonium acetate (DAA) adjusted to pH 5 in water; mobile phase B was comprised of methanol. All data was normalized using DNA concentration obtained from cell pellets.

Results: Initial results indicate substantial decrease in the levels of intermediates of the oxidative PPP branch and increased levels of intermediates of non-oxidative branch and glycolysis in cells with G6PD knockdown. In contrast, we found an increase in the intermediates of oxidative branch in cells with the knockdown of TKT. The decrease in PRPP, the final product of both branches of PPP, was found only in the case of G6PD knockdown.

Conclusions: The results indicate that there is a competitive nature between the oxidative and non-oxidative branches of the PPP for glucose metabolism. Further work is needed to determine if the accumulation of metabolites is a result of increased metabolic rates, or caused by decreased enzymatic rates occurring downstream in the respective pathways.
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