Selected lectures and illustrations

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Caroline Abadie, MD; Centre Hospitalier Universitaire de Rennes; Rennes, France

Long-term prognosis of resected pancreatic neuro-endocrine tumors (PNETs) in von Hippel-Lindau is favorable and not influenced by small tumors left in place
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Martin Walz, MD; Kliniken Esse-Mitte; Essen, Germany

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Samuel Sommaruga, MD; Yale University; New Haven, Connecticut, USA

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Jean-Michel Correas, MD; Service de Radiologie; Hôpital Necker; Paris, France
Basic and clinical research in pheochromocytoma

Karel Pacak, MD; National Institutes of Health; Bethesda, Maryland, USA

Among endocrine tumors, pheochromocytomas (pheos) have the highest percentage (55%) caused by known genetic mutations. Inherited mutations represent 35% of all pheos. Although 18 separate pheo susceptibility genes have been found, common themes arise with these mutations. For example, abnormal regulation of HIF-α occurs as a result of a number of these mutations.

Since 2000, there has been a shift from looking for catecholamines in the bloodstream to testing for metanephrines in the blood to diagnose and follow pheochromocytomas. Catecholamines are now outdated as a measurement tool.

Methoxytyramine is a new biomarker for diagnosis of pheos, and was introduced in 2011 as a measurement tool. However, only about 17% of VHL patients with pheos secrete methoxytyramine. An increase in methoxytyramine may significantly precede catecholamine release. This may be the reason that some patients have 6 – 7 cm tumors upon diagnosis.

The most recent diagnostic tool for pheos is metabolomics, the study of chemical processes involving metabolites. Using a “mass spectrometer”, a highly sensitive instrument that can identify and quantify specific proteins, protein expression levels in tumors can be assessed and the profile of protein expression defined by this tool may be a more useful way of diagnosing and categorizing tumors than the “old fashioned” staining of tumor tissues.

The Cancer Genome Atlas (TCGA) study at NCI.NIH in Bethesda is really a consortium of dozens of institutions around the world. TCGA is working on a comprehensive genomic characterization of pheos, which ultimately will allow us to understand how and why these tumors develop.

Metabolic vulnerabilities of VHL-deficient cells highlight novel targets for therapy

Othon Iliopoulos, MD; Harvard Medical School; Boston, Massachusetts, USA

Cancer cells and cells deprived of oxygen (“hypoxic cells”) redirect glucose to lactate (similar to lactic acid production due to exercise).

The next step in metabolism is the TCA (or Krebs) cycle where citrate gets converted into a variety of metabolites to generate energy for the cell. Normal metabolism uses oxygen and produces carbon dioxide. In VHL deficient cells, the Krebs cycle is blocked, and actually runs “backwards” to produce specific lipids (or fats) and certain components of the DNA backbone called pyrimidine nucleotides. Fueling this backwards movement of the Krebs cycle is glutamine, the most common amino acid (the building block of proteins). If you block glutamine breakdown by inhibiting glutaminase, the “gatekeeper” enzyme into this reverse cycle, you can selectively suppress growth of VHL deficient cells.

Conclusion:

VHL-deficient cells are sensitive to glutaminase inhibition in vitro and in vivo by systemic administration of glutaminase inhibitors to mice with RCC xenografts (tissue grafted from another species), growth of RCC cells was suppressed. This exciting discovery has led to the development of a clinical trial testing glutaminase inhibitors in humans.

Transcriptional suppression of PPAR gamma coactivator 1 alpha (PGC1) by hypoxia-inducible factors inhibits mitochondrial biogenesis in clear cell carcinoma

Edward LaGory, PhD & Amato Giacca, PhD; Stanford University; Stanford, California, USA

Over 75% of clear cell renal carcinomas (ccRCC) show loss of the tumor suppressor gene, VHL. This loss makes the cell’s oxygen-sensing defective and suppresses a vital coactivator of metabolic genes called peroxisome proliferator-activated receptor gamma coactivator 1, or PGC1. Loss of this gene is
associated with the cancer cell's tendency not to use the Krebs cycle to generate energy for the cell. When the expression of PGC1 is restored to VHL-deficient ccRCC cells, oxygen consumption becomes similar to cells with functional VHL. This also decreased cell growth. This research suggests that stimulating PGC1 may be a treatment for ccRCC.

Transcriptomic analysis of clear cell renal carcinoma (ccRCC) and pheochromocytomas (pheo)

W. Kimryn Rathmell, MD, PhD; UNC Lineberger Cancer Center; Chapel Hill, North Carolina, USA

Studies at UNC of ccRCC demonstrate that in both inherited and sporadic ccRCC, gene expression patterns can provide information that may predict the potential aggressivity of specific tumors. One of the challenges facing researchers is that this gene expression pattern may vary even within a particular tumor. However, core features of VHL mutation are maintained. Small tumors have less variety than larger tumors, and even glucose uptake can vary within a single tumor, which is being tested with techniques like a Positron Emission Tomography, or PET scanner. Dr. Rathmell’s group is continuing to study the impact of this intratumoral heterogeneity on our ability to both predict tumor behavior and our ability to treat these tumors when they spread.

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Role of VHL in ciliogenesis

Wilhelm Krek, PhD; Institute of Molecular Health Sciences; Zurich, Switzerland

The primary cilium is a non-moving projection found on the surface of most cells, which is responsible for the cell's ability to "sense" what is going on outside it, and is one of the gatekeepers of the cell's entry into division, or mitosis. VHL was shown to be an important regulator of the primary cilium under certain conditions. Dr. Krek's group looked for factors that were associated with this changed behavior.

They found that altered levels of hypoxia inducible factor (HIF) in cells results in a cascade effect that misregulates other proteins involved in cilia maintenance. You need a certain level of HIF1α in the cell to permit a cilium to form, and knocking down HIF1α prevents normal cilia formation.

The inconsistency in more advanced forms of RCC is that we see selective upregulation of HIF2α and down regulation of HIF1α expression, and that HIF1α may actually be a tumor suppressor. The question that Dr. Krek's work raises is whether HIF1α deletion in RCC might deprive renal cancer cells form the option to build a cilium – is this an aspect of the HIF1α tumor suppressor function?

Aurora kinase and VHL in kidney cancer

Yannick Arlot, PhD; Institute de Génétique et Développement (IGDR); Rennes, France

Cancer is a defect in cell cycle progression. Clear cell kidney cancer (ccRCC) exhibits cell division defects. One is dysregulation of the Aurora-A kinase, identified as essentially an oncogenic protein in several human cancers. A study conducted at IGDR demonstrated that the VHL and Aurora-A proteins physically interact in a test tube and may functionally interact in ccRCC tumors. This interaction appeared more prominent in more aggressive kidney cancers and in cancers where VHL protein is expressed. This knowledge indicates that modulating Aurora-A kinase may be a useful therapeutic approach in patients with certain types of advanced ccRCC.

Identification of molecular drivers of human hemangioblastoma

Mianen Sun, PhD; MD Anderson Cancer Center; Houston, Texas, USA

A study conducted at MD Anderson found that HNF1B is lost in human hemangioblastomas. The investigators then examined the effects of Hnf1b loss in mice. They found that “knocking out” Hnf1b caused chromosomal instability and a dramatic increase in vascular endothelial growth factor expression. There was also abnormal cell division, similar to tumor growth. The loss of HNF1B when combined with mouse VHL loss induced endothelial cell vasculogenesis, or blood vessel growth. The loss
of HNF1B could be one of the major drivers of the hemangioblastoma disease phenotype in patients with VHL disease.

**Role of mutant von Hippel-Lindau on angiogenesis**

Alexandra Arreola, PhD; UNC Lineberger Cancer Center; Chapel Hill, North Carolina, USA

Variance in VHL disease “phenotype” or pattern of disease expression is due to lack of tight regulation of gene activity, especially in certain mutations such as “hotspot” R167Q mutation in VHL type 2B. This variation retains partial regulation of HIF (hypoxia inducing factor), leading to variations in the VHL disease between patients with VHL type 2B.

VHL type 2B in a mouse model was tested at UNC. These results suggest that a finely tuned balance of HIF factors along with moderate differences in VHL protein levels can lead to significant and distinct defects in angiogenesis (blood vessel formation).

**Summary:**
- VHL loss leads to complex alterations in angiogenesis
- Even small increases by VHL2B/+ may be enough to affect proper angiogenesis
- VHL 2B mutations result in further disruption of proper angiogenesis: increased branching, aberrant sprouting of blood vessels, and altered NOTCH (a protein that spans the cell membrane) signaling

A better understanding of how various VHL mutations affect blood vessel formation and other aspects of tumorigenesis will allow us to fine-tune our treatment approaches for patients with VHL disease.

**L-2-Hydroxyglutarate: an epigenetic modifier and putative oncometabolite in renal cancer**

Sunil Sudarshan, MD; University of Alabama; Birmingham, Alabama, USA

The metabolite 2-hydroxyglutarate (2HG) is elevated in renal cell carcinoma (RCC). 2HG may be an epigenetic modifier (alters gene expression) and oncometabolite (small molecule produced by normal metabolism that has a role in tumor formation if it accumulates) in RCC.

**Conclusions:**
- L-2HG is elevated in RCC
- Loss of L2HGDH gene promotes L-2HG elevation
- L-2HG is an epigenetic modifier, which means it modifies the machinery that influences gene expression.
  - It promotes 5hmC (influences regulation of gene expression) loss
  - It promotes a DNA hypermethylator (produces gene expression changes that contribute to tumorigenesis) phenotype
- L2HGDH has tumor suppressor activity in RCC
- L-2HG is a putative oncometabolite

This knowledge can lead to new therapies that specifically target tumor cells and spare normal cells.

**Next-generation sequencing allows the identification of VHL mosaicism: study of a panel of 16 patients**

Pascal Pigny, PhD; Centre Hospitalier Régional Universitaire (CHRU); Lille, France

Mosaicism describes an individual who has developed from a single fertilized egg, and has two or more populations of cells with distinct “genotypes”, or genomic makeup. Mosaicism usually arises when a mutation occurs during the initial few rounds of cell division after fertilization, so that some of the cells in the body have that mutation, and others do not.

The prevalence of mosaicism in VHL is probably underestimated since identification by conventional means requires a complicated and labor-intensive cell enrichment step. At CHRU, Next generation sequencing (NGS) is used in order to detect...
mutational events that occur at a frequency of 1%. With this technique, you can pick up one mutated cell out of 100 cells of the same subtype.

A study to find VHL mosaicism using next generation sequencing was conducted:

- Tested on 4 controls with known mosaicism, and was shown to work
- Then tested 16 patients who tested negative for VHL by traditional Sanger sequencing or multiplex polymerase chain reaction (PCR) testing
- Six of these patients had classic VHL lesions, 10 had some features that suggested VHL
- Three of these 16 patients were identified as VHL mosaics; of these three patients, two had classical VHL; the third had retinal hemangioblastomas

Conclusions:

Next generation sequencing allows the detection of mosaic VHL mutations in patients. The frequency of mosaicism found in this study was higher than expected at 18.7%. This illustrates the usefulness of next generation sequencing for detection of VHL mosaic mutations.

### Diagnosis and clinical management of VHL disease

**Giuseppe Opocher, MD; Veneto Institute of Oncology; Padova, Italy**

The clinic in Padova began to work with VHL patients in the late 1990s and is currently following 240 individuals (1500 visits total).

### Pathology of VHL disease

**Alexander Vortmeyer, MD, PhD; Yale University School of Medicine; New Haven, Connecticut, USA**

This presentation focuses on the principles of neuroectodermal and mesonephric tumorigenesis. There are three types of embryonic tissue: the Ectoderm (gives rise to the skin and nervous system), the Mesoderm (gives rise to mucous membranes and digestive glands), and the Endoderm (gives rise to muscle, bone, and blood). Lindau felt that the third month of embryonic development was important to tumor development. We see microscopic changes in developmentally arrested structures that are VHL deficient. Each embryonic tissue is related to specific VHL tumors:

#### Neuroectodermal
- Hemangioblastomas in nervous system (CNS, eye, ear)

#### Mesonephric
- Clear cell carcinomas in the kidneys
- Cystadenomas in the epididymis

#### Neuroendocrine
- Neuroendocrine tumors in the pancreas

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<tr>
<th>Organ</th>
<th>Earliest Age of Onset</th>
<th>Age 10% First Disabled</th>
<th>Disability Criteria</th>
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<tr>
<td>Kidney</td>
<td>25 years</td>
<td>60–69 years (4%)</td>
<td>Dialysis</td>
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<tr>
<td>Epididymis</td>
<td>16 years</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Pancreas</td>
<td>12 years</td>
<td>45–50 years (6%)</td>
<td>Substitutive therapy</td>
</tr>
<tr>
<td>CNS</td>
<td>12 years</td>
<td>20 – 25 years</td>
<td>Sensory and/or motor problems</td>
</tr>
<tr>
<td>Adrenal</td>
<td>7 years</td>
<td>31 – 39 years</td>
<td>Substitutive therapy</td>
</tr>
<tr>
<td>Eyes</td>
<td>6 years</td>
<td>19 – 29 years</td>
<td>Blindness (one or both eyes)</td>
</tr>
<tr>
<td>Ear</td>
<td>unknown</td>
<td>47 – 50 years</td>
<td>Hearing loss (one or both ears)</td>
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CNS lesions in the Padova patient group result in the highest rate of disabilities peaking at about 34% by the age of 50-59 years. Disability from retinal lesions (monocular and binocular blindness) begins as early as 0-9 years of age and peaks at about 21% by age 50-59%. The kidney has the lowest rate of disability (as measured by the need for dialysis), peaking at about 3% by the age of 30-39.
• Pheochromocytomas in the adrenal glands and paragangliomas outside of the adrenals

HIF (Hypoxia Inducible Factor) is common to all VHL tumors. VHL deficiency (due to VHL mutations) leads the cell to believe that it is hypoxic and needs oxygen. This leads to angiogenesis (blood vessel formation) in an attempt to supply more oxygen to the cell. The VHL mutation that causes VHL disease is the “first hit.” In all issue types, another VHL mutation, the “second hit” is necessary, but insufficient to induce tumorigenesis. The second hit produces developmentally arrested VHL-deficient cells (DASEs). The “third hit” is the switch from DASE to tumor cell (tumorigenesis). More studies are needed to analyze and better understand the morphology and molecular processes involved in VHL tumorigenesis.

A comprehensive study of germline mutations in the VHL gene reveals the importance of precisely-tuned dysregulation of the hypoxia pathway in oncogenesis

Betty Gardie, PhD; Ecole Pratique des Hautes Etudes; Paris, France

A study of naturally occurring VHL mutations in families in France validated the recently proposed “continuum” model of tumor suppression. The study was able to demonstrate that the complex pattern of disease manifestations seen in VHL is perfectly correlated with the level of pVHL (VHL protein) dysfunction in hypoxia (low oxygen level) signaling pathways.

The study looked at 30 genes directly regulated by VHL or HIF2α. The findings support a continuum model in place of the “two hit” model and explain why polycythemia is sometimes associated with VHL: it is on the continuum of VHL mutations.

The continuum model correlates well with the risk of both pheochromocytomas and renal cell carcinomas. Further study is needed to see if it correlates with hemangioblastoma formation.

Gallium DOTATATE PRT/CT detects primary neuroendocrine tumors and metastases in patients with von Hippel-Lindau

Samira Sadowski, MD, National Cancer Institute, Bethesda, Maryland

This study was conducted to determine the utility of PET (positron emission test) radiopharmaceuticals in detection of primary and metastatic neuroendocrine tumors (NETs) in patients with VHL. Approximately 17% of VHL patients will develop NETs.

The study looked at 13 patients diagnosed with VHL and NETs. The PET scan with Gallium DOTATE found 6 patients with new metastatic lesions. In 5 of these patients, this result led to changes in their recommended treatment.

Screening and diagnostic aspects of endolymphatic sac tumors (ELSTs)

Marie Louise Mølgaard Binderup; University of Copenhagen; Copenhagen, Denmark

ELSTs occur in up to 16% of VHL patients and 11–30% of those are bilateral. Most patients (96%) are not diagnosed until they have symptoms: hearing loss (91%), ringing in the ears (64%), or dizziness (52%). These symptoms are not always cured by surgery, so the challenge is to diagnose at the earliest possible stage.
Hearing loss from ELSTs can be gradual or sudden:

- Gradual hearing loss
- Sudden hearing loss

The recommended screening tests for patients with VHL include regular MRIs to detect ELSTs as early as possible. However, not all VHL patients have access to these MRI screening tests. A possible alternative is a hearing test to detect ELSTs.

An international collaborative study was conducted to learn how audiometry testing can be used for ELST screening in VHL patients. The first step was to determine hearing characteristics of VHL patients (over age 15 years). Patients with and without diagnosed ELSTs were included. The researchers in the study investigated whether any hearing patterns correlated with ELST development. Researchers looking at hearing test results did not see the inner ear MRIs, and the researchers looking at the MRIs did not see hearing test results.

The study found that 44% of VHL patients had poorer hearing than expected for their age. Low frequency hearing loss has been associated with both VHL and Meniere’s disease. This study compared hearing loss patterns with MRI-visible ELSTs and found only two patients with both. The next step in this ongoing study is to find if the other patients with low frequency hearing loss go on to develop ELSTs.

**Pheochromocytoma and pregnancy in VHL patients**

Jacques Lenders, MD, PhD; Radboud University Medical Center; Nijmegen, the Netherlands

Although pheochromocytomas (pheos) are rare in the general population, they occur in approximately 20% of the VHL patient population. The average age at diagnosis for VHL patients is 25–30 years old meaning that the patient may be pregnant or trying to conceive when diagnosed.

It is important to differentiate between symptoms of a pheo during pregnancy and the high blood pressure that appears in some women during pregnancy (pre-eclampsia):

- **Differential diagnosis pheochromocytoma vs pre-eclampsia**
  - **Pheochromocytoma**
    - more likely than pre-eclampsia
  - **Pre-eclampsia**
    - more likely than pheochromocytoma

Diagnostic testing for pheos is the same during pregnancy as in patients who are not pregnant. However, scans to locate the pheo should not involve radiation; MRI is the best choice as ultrasound is not sensitive enough. If a pheo is diagnosed in a pregnant patient, surgery should be scheduled before 24 weeks or during a C-section, or after the birth.
Genetic counseling on VHL
Ignacio Blanco, MD; Germans Trias Hospital; Barcelona, Spain

Genetic counseling is a communication process that goes beyond performing a genetic test. It is a process, not an act. Counseling must be differentiated from giving information or having a discussion.

Counseling is a foreign concept in many countries:
- Need to address the implications of information
- Help people understand and adapt to the medical, psychological, and familial implications of disease

Counseling integrates three areas:
- Risk assessment
  - Need family pedigree
  - Risk communication – uses graphics from vhl.org wellness section
- Education and health promotion
  - Use knowledge-sharing, not traditional teaching to transmit information
  - Encourage use of handbooks, such as those from the VHLA
- Facilitation of decision-making

The emotional aspects of VHL may influence the sense of self, especially for children and young adults. It is important to encourage those with VHL to disclose information to those who would be affected. Counseling should include the entire family (adults and age-appropriate children) so that everyone receives the same information at the same time. The emphasis in VHL has shifted from reaction to prevention and counseling must take a central role.

Education about VHL is important:
- Increasing the knowledge about VHL improves patient’s autonomy and modifies attitudes about the disease
- Knowledge about carrier status enables early diagnosis and intervention
- Knowledge decreases uncertainty and anxiety and assists in making decision about lifetime plans

Conclusions:
- Genetic counseling, including risk assessment, health education and counseling, is an essential aspect of VHL care
- The laboratories offer technical knowledge and expertise while genetic counseling can translate this knowledge into practice to help those affected make more informed decisions. Having one without the other is an inefficient way forward
It is important to achieve a balance between giving information and discussing the implications so that people do not leave confused and unclear about the main issues for their situation.

**Von Hippel-Lindau disease in childhood: a retrospective French series**
Caroline Abadie, MD; Centre Hospitalier Universitaire de Rennes; Rennes, France

This is a review of the spectrum of VHL disease in 157 children under age 16 from 132 families in France. A French clinical database for VHL was established in 1990 and includes data for 1147 patients. To our knowledge, this report looks at the largest number of VHL patients diagnosed as children. VHL genetic testing is conducted in suspected children beginning at age 5 years.

The average age that the first manifestation of VHL was seen in this population was 13 years old. The average age that the first retinal hemangioblastoma was found was 13 years, the first pheo/para was 11 years, and the first CNS lesion was 14 years. Lesions in multiple organs were present in 73% of the patients. Early childhood testing and screening is needed to manage VHL lesions before they become symptomatic. In this study, retinal hemangioblastomas and pheochromocytomas are the first presentations of VHL.

**Long-term prognosis of resected pancreatic neuro-endocrine tumors (PNETs) in von Hippel-Lindau is favorable and not influenced by small tumors left in place**
Louis de Mestier, MD; University Hospital; Reims, France

This study looked at two groups of patients with PNETS, one group with VHL, and one without. The groups were demographically similar except for age: VHL patients were an average of 20 years younger age: 36 vs. 56 years. VHL patients had a better long-term outlook than those with sporadic PNETs and we found that small PNETS left in place are at low risk for growth or metastasis.

**Conclusions:**
- Low recurrence rate following VHL-PNETS resection
- Close follow-up if there are predictive factors of an unfavorable course
- Better prognosis than sporadic PNETS

**State-of-the-art of the therapeutic approaches**
Eric Jonasch, MD; MD Anderson cancer Center; Houston, Texas, USA

The current standard of care for management of VHL lesions involves the surgical removal of renal cell carcinomas, pancreatic neuroendocrine tumors, and central nervous system hemangioblastomas that are at risk for metastasis, causing significant organ damage, or causing symptoms. Retinal lesions are most often treated with laser ablation. An understanding of the molecular biology of VHL is useful in understanding current approaches and possible future treatments.

**Coming Up With A Cure:**
Many Layers of Knowledge are Needed!

- Identification of the VHL Gene
- Description of VHL Protein Function
- Identifying and Characterizing Additional Genes Disrupted in VHL Disease
- Development of Relevant Model Systems

**VHL- A Regulatory Hub**

- Extracellular Matrix Control
- p53 Regulation
- Primary cili Function
- Angiogenesis

The VHL protein is necessary for many normal cell functions:
Mutations in the VHL gene sequence lead to VHL protein that is absent or does not function correctly. These malfunctions have “downstream consequences” which are points that treatments could target for repair.

Over 1/3 of VHL mutations (hereditary or sporadic) are missense mutations, therefore some functionality of the VHL protein may remain. A full-sized protein remains, unlike other VHL mutations (nonsense, microdeletion, deletion insertion, splice). What this means is that you have a full sized protein that can possibly be fixed.

Other mutations are important in forming hemangioblastomas: HNF1B; and Renal Cell Carcinoma: SETD2, PBRM1, BAP 1. Understanding the interaction of these genes with VHL is critical to developing an accurate model of VHL disease. Once there is a good model of VHL disease, targeted treatments can be developed.

Current therapies that can “round off the edges” of biology arising from VHL deficiency, and may in some cases be helpful

• Only by characterizing the additional genomic drivers of renal cell carcinomas, hemangioblastomas, pheochromocytomas, and NETs will we be able to devise appropriate model systems that can help develop curative therapy.

• We need to do more basic science with VHL to find a cure.

A zebrafish model to study and therapeutically manipulate hypoxia signaling in tumorigenesis

Rachel Giles, PhD; University Medical Center; Utrecht, the Netherlands

Recent advances in DNA sequencing technology, such as next generation sequencing (NGS), now allow research labs to sequence multiple genes quickly at a reasonable cost. This allows the range of phenotypes to be determined for known human diseases.

The zebrafish is an excellent model for VHL as homozygous (2 VHL gene mutations) fish can be used. Unlike humans, the VHL homozygous deleted zebra fish embryos survive. These VHL fish are easily identified as they have spots instead of stripes. Their kidneys form many cysts, and without treatment, they
die from kidney failure. If VHL is added into the defective kidney, however, normal function is restored.

The zebrafish VHL model suggests that the primary kidney defect is due to cell polarity and vesicle “trafficking” in the renal epithelium. Zebrafish with VHL mutations can be used for many research studies including:

- Biomarker development
- Investigate 2nd hits
- Personalize medicine

Improving oral TKI treatment for ccRCC

**Mouse models of cooperative tumor suppression in clear cell renal cell carcinoma**

Ian Frew, PhD; Zurich Center for Integrative Human Physiology, University of Zurich; Zurich, Switzerland

Clear cell renal cell carcinoma (ccRCC) is one of the few major human cancer types for which there is no identical mouse model. This is because ccRCC is not a single change in the kidney tissue. Mice can form similar kidney cysts when they have both VHL and Pten deletions. Both act as tumor suppressor genes. The cysts that are formed have reduced activity of the primary cilia. The combination of the VHL mutation and the loss of cilia lead to cyst formation.

Hypoxia inducible factor is the next step in ccRCC due to effects on renal epithelial proliferative homeostasis. HIF1α acts as a tumor suppressor, and HIF 2α is oncogenic, promoting tumor growth. Knocking out the VHL gene leads to an increase in both HIF1 and HIF2.

Based upon the VHL mouse model, a progressive model for ccRCC in humans with VHL is proposed.

**Correction of folding defects of oncogenic pVHL by small molecules leads to restoration of its function in vitro and to development of a corresponding animal model for the disease**

Daniel Segal, PhD; Tel Aviv University; Tel Aviv, Israel

Mathematic analysis predicts that VHL mutations disrupt the normal folding of the pVHL protein. The pVHL tumor suppressor protein is intrinsically unstable and loss of stability reduces the ability of pVHL to bind to HIF-1α. Oncogenic (tumor producing) mutations result on pVHL misfolding and loss of function.

Arginine (amino acid found in meat and dairy foods) was found to be a potent re-folder of pVHL. This was tested in vitro, then in vivo using fruit flies. Arginine can restore pVHL function of binding HIF-1α. Tel Aviv University developed a transgenic strain of fruit flies expressing mutant forms of pVHL to facilitate in vivo testing of small molecules capable of re-folding pVHL. This model is also being tested in renal carcinoma cells harboring VHL mutations.

**Conditional inactivation of the mouse von Hippel-Lindau tumor suppressor gene results in widespread hyperplastic, inflammatory, and fibrotic lesions in the kidney**

Tien Hsu, PhD; Boston University School of Medicine & National Central University; Taiwan

Cancer is usually an adult disease, but the seed is planted early.

- Liver hemangiomas and clear cell lesions are seen BEFORE kidney lesions. Are these metastatic?
- Severe fibrosis in the kidneys is seen before metastasis occurs; therefore, pre-cancerous lesions are fibrotic.
Two VHL phenotypes (VHL mutant phenotypes are dependent on HIF-1, but not HIF-2):

- Phenotype 1: clear cell lesions in the liver
- Phenotype 2: polycythemia (increased number of red blood cells)

A review of studies that included VHL patients indicate that inflammation stimulates clear cell renal cell carcinoma (ccRCC) development. These studies, when taken together, suggest that VHL mutant cells induce an inflammatory microenvironment, which in turn precipitates the start of ccRCC.

**Cutting edge in systemic treatments of neuroendocrine tumors (NETs)**

Jaume Capdevila, MD; Vali d’Hebron Institute of Oncology; Barcelona, Spain

Treatment goals of pancreatic NETs must balance: 1.) Tumor growth control, 2.) Tumor burden, and 3.) Quality of life. Chemotherapy is suitable only for large, aggressive NETs.

Targeted therapy (of somatostatin receptors on the NET cellular surface) has only been effective on tumors within the pancreas. These therapies include everolimus and sunitinib. Lanreotide is currently being tested for effects on NETs located outside the pancreas. Surgery is still the first choice of treatment for localized NETS, and can be an option in metastatic disease to reduce tumor burden and symptoms.

**Conclusions:**

- The increasing knowledge of genomic landscape of NETs is showing a spectrum of different tumors under the same umbrella. This knowledge should be useful for a better selection of patients to be treated with targeted agents.
- Targeted agents have changed the scenario of therapy for advanced NETs, but predictive/prognostic biomarkers are still far to be included in prospective clinical trials.

**Therapeutic markers of antiangiogenic drugs**

Cristina-Rodriguez-Antona, MD; Hereditary Endocrine Cancer Group; Centro Nacional Investigaciones Oncológicas; Madrid, Spain

Angiogenesis is a central hallmark of cancer and the first angiogenic treatment (sorafenib) became available 10 years ago. However, there are several issues with drugs that target angiogenesis: relatively short-term effects, context-specific efficacy (early stage vs. metastatic), and, for VHL, no markers available to guide clinical selection of drugs.

Renal cell carcinoma is a prototype vascular endothelial growth factor (VEGF)–driven tumor. Sunitinib and pazopanib are the most common treatments with positive responses in 25 to 40% of patients. However, up to 20 to 25% of patients obtain no benefit from these drugs. Predictive markers are needed to determine which patients will benefit.

Another challenge in treating RCC is that the cells within the tumor differ, impacting drug activity and leading to eventual drug resistance. Part of the tumor microenvironment, single nucleotide polymorphism (SNP), remains constant and may provide a predictive marker.

**Conclusions:**

- Antiangiogenic drugs are used to treat many cancers, however, several challenges limit their use
- Several makers have been suggested as predictors of resistance to antiangiogenic agents, However, validation in large independent series is necessary
- Acquired resistance is a common event, which might be promoted by high intratumor heterogeneity (cell variation within a single tumor)
- Malignant pheochromocytomas with high expression of VEGF pathway components (e.g., SDH-mutations) may benefit from antiangiogenic therapy
- The use of antiangiogenic agents will increase in the future. Thus it is critical to define mechanisms of resistance and identify predictive markers to improve clinical management
Therapeutic challenges in ocular involvement
José García Arumi, MD; Hospital Universitario Valle Hebrón; Barcelona, Spain

VHL eye lesions are usually first seen between ages 10 to 30 years. There are multiple lesions found in 30% of patients when they are first seen. The tumor mass can appear either red or white. Any exudates (fluid) from lesions will disappear after laser treatment or cryotherapy. Most lesions are seen in the peripheral retina, away from the center of vision. Capillary papillary hemangioblastomas (on the optic nerve) and juxtapapillary (around the optic disc) are relatively rare, but are challenging to treat and have a higher rate of visual loss.

Exploiting synthetic lethality in kidney cancer to target the loss of the von Hippel-Lindau tumor suppressor gene
Sandra Turcotte, PhD; Atlantic Cancer Research Institute; Université de Moncton; New Brunswick, Canada

Regulation of autophagy (cellular process that coordinates the degradation of proteins, lipids and organelles during both basal and cellular or environmental stress conditions) as a cancer therapy is an approach that only kills off cancer cells missing at least one VHL-functional gene. Up to 85% of renal cells in VHL have no functional VHL gene. The study we conducted was designed to investigate how a small molecule selectively inhibits and kills cells with no functional VHL gene.

This small molecule is known to be HIF-independent and modulates autophagy. Autophagy is important for selective cytotoxicity (poisoning cells). This small molecule may also target lysosome (waste disposal mechanism of a cell) degradation.

This research reveals a role for VHL in autophagy and lysosomal degradation that could be used as a therapeutic approach to treat VHL RCC. There is no toxicity to non-cancer cells.

Therapy for sporadic and von Hippel-Lindau-related hemangioblastomas of the central nervous system
Sven Gläsker, MD; Department of Neurosurgery; Freiburg University Medical Center; Freiburg, Germany

Patients with VHL need a different, individualized approach to treatment of hemangioblastomas in the central nervous system. Outcome varies with location of the lesion. Medullary hemangioblastomas in the brain result in temporary symptoms (20%), permanent deficits (5%), and improvement with treatment (20%). Cerebellar hemangioblastomas rarely cause a deficit and good improvement is seen for cystic tumors.

The experience at Freiburg University Medical Center indicates that there are no alternative treatments as effective as surgery, however in case of inoperable tumors and for patients who cannot tolerate an operation, radiotherapy experimental chemotherapy, decompression (to reduce pressure in the brain), or embolization (blocking a blood vessel) may be considered.

Neurosurgery management of hemangioblastomas in difficult locations: Brain stem and spinal root hemangioblastomas – role of neuronavigation and intraoperative neurophysiological monitoring
José Maria de Campos, MD; Department of Neurosurgery; VHL Unit; Fundacion Jiménez Díaz; Madrid, Spain

CNS hemangioblastomas are the most frequent tumors in VHL patients. Brainstem and spinal root hemangioblastomas are less frequent and well-known than cerebellar and spinal cord lesions.

Symptoms specific to brainstem lesions include: dyspnea (difficulty breathing), coughing, and hiccups. A poorer prognosis is associated with: previous surgeries in the posterior fossa (space in the skull near the brainstem) surgeries, no cystic tumor component, and poor pre-operative function. Surgery is recommended due to tumor size (1-1.5 cm) and/or at the beginning of symptoms. The surgeon will try to locate and remove all tumors in the area, to reduce subsequent hazardous procedures.
Spinal root hemangioblastomas, located where spinal nerves emerge from the spinal cord before the anterior and posterior portions join to become the complete spinal nerve). Spinal root hemangioblastomas need to be removed while they are small, even though there may be no symptoms. The operation is much simpler and less risky while these tumors are still small. Neuronavigation and intraoperative neurophysiological monitoring can help to safely remove these tumors. These computer images assist the surgeon when operating in this restricted area.

**Radiosurgery in CNS hemangioblastomas. When and how?**

Maria Elena Kusak, MD; Department of Neurosurgery, Radiosurgery Unit; Hospital Ruber Internacional; Madrid, Spain

Radiosurgery has advanced to include spinal as well as cranial lesions. It may be administered in up to 5 sessions; it is not just a single session procedure. Radiosurgery is a minimally invasive treatment in which several lesions can be treated in the same session. However, safety and long term efficacy are still to be demonstrated.

There are concerns about radiation, even the very focalized radiation used in radiosurgery. It is unknown if it may induce malignant transformation in preexisting tumors, or promote the development of new tumors. In VHL, new tumors may be mistaken for the natural history of the disease. There is no specific information about metastatic lesions in the brain or spine; however, there have been excellent results in non-VHL patients.

Radiosurgery in VHL patients should be performed only if the lesion is inoperable or the patient is unfit for surgery. Radiosurgical follow-up is still short-term; expect some long-term issues.

**Therapeutic strategies for central nervous system hemangioblastomas in von Hippel Lindau disease**

Hiroshi Kanno, MD; Department of Neurosurgery, Yokosuka City Hospital; Yokohama University; Yokohama, Japan

Among the VHL patients seen at Yokosuka City Hospital, missense mutations are the most common VHL mutation in patients with CNS hemangioblastomas (the most common VHL mutations overall are frameshift and nonsense mutations). Missense mutations, unlike frameshift or nonsense mutations and deletions may have a partially functioning VHL protein.

Surgery is the first choice, except for brainstem lesions. In most cases, the outcome achieved is excellent. Out of 42 patients operated on, 81% were Grade 0 post-surgery, 10% were Grade 1, 5% were Grade 2, 1 patient was Grade 3, and one patient was Grade 4. The Eastern Cooperative Oncology Performance Status (ECOG PS) score is from 0 (perfect health) to 5 (death).

Stereotactic radiosurgery (SRT) is the second choice option for VHL patients. Eleven VHL patients who needed SRT were seen. Over half of these (5) had no change in their tumor following SRT. The tumors shrank in 3 patients and another 2 patients had their tumors disappear. The other 3 patients had swelling around their tumors after SRT.

**Minimally-invasive surgery for pheochromocytomas and retroperitoneal paragangliomas**

Martin Walz, MD; Kliniken Esse-Mitte; Essen, Germany

Surgery is the treatment choice for pheochromocytomas and paraganglioma lesions in VHL. The development of minimally invasive surgical techniques in the 1990s has revolutionized surgical treatment for these tumors. For example, the average length of hospital stay has dropped from 9 days with an anterior open procedure, to 3 days with a minimally invasive procedure.

Minimally invasive surgery allows surgeons to:

- Operate on multiple tumors in a single operation (up to 8 tumors)
- Have two surgical teams work together to remove tumors in multiple sites at the same time
- Operate retroperitoneally (through the back) in most cases as this is a shorter route to the tumors
- Shorten operating time – average is 50 minutes
- Reduce average hospital stay to 3 days
- Reduce pain – 50% will need no pain meds (if peritoneum is not touched, there is little to no pain)
- Use tiny incisions leave minimal to no scarring
- Preserve cortical adrenal function in over 90% of patients with bilateral tumors (no post-op steroids needed)

This technique should only be performed by experienced teams. Training is given around the world, including the U.S.
Surgery for endolymphatic sac tumors (ELSTs) and auditory rehabilitation
Carlos Cenjor, MD; ENT Department, Fundacion Jiménez Diaz; Madrid, Spain

Surgery for ELSTs may preserve hearing if the surgeon is able to avoid damage to the labyrinth of the inner ear. Bone anchored hearing aids are a good choice for hearing loss in one ear.

If hearing is reduced in both ears to less than 50% speech discrimination, cochlear implants may be tried.

Patients must be informed that a standard MRI cannot be used following an implant and that the implant itself will block certain areas of the brain from view.

Challenges in renal cell carcinoma (RCC) surgery
Jean-Jacques Patard, MD, PhD; Centre Hospitalier Universitaire (CHU); Bicêtre, France

Most VHL associated RCCs exhibit a consistent, predictable growth pattern and are unlikely to metastasize when smaller than 3 cm. The treatment goal is to remove renal tumors before they reach to metastatic stage while preserving normal kidney function.

It is important to limit ischemic damage to the kidney during surgery. There is a longer ischemic time with laparoscopic surgery than with open surgery. Robotic surgery is an emerging choice and ablative techniques are becoming standard in second and third surgeries on the same kidney. Ablation is starting to be considered for first surgeries. Open surgery is still the best choice for removal of multiple tumors.

Promising new techniques include minimally invasive procedures, including robotic surgery. A hybrid approach, where robotic partial nephrectomy is combined with radiofrequency ablation or cryoablation is becoming a second or third choice alternative.

Mesonephric carcinogenesis in VHL disease
Samuel Sommaruga, MD; Yale University; New Haven, Connecticut, USA

Recent autopsy studies of tissue from the spinal cord, cerebellum, and epididymis show that the “second hit” VHL mutation thought to cause VHL tumors actually produces an abundance of developmentally arrested structural elements (DASES). These DASES have the potential to develop into tumors, but most do not. Now the question becomes what is the “third hit” in VHL? Our study concludes that the “third hit” results in intratubular clear cell neoplasia in the kidney. The VHL kidney appears to present a mosaic of VHL-competent and VHL-deficient (clear) cells with the potential to progress into carcinoma.

Conclusions:
- VHL kidney may reveal thousands of microscopic, potential cancer precursor structures (DASEs)
- A small subset of VHL-deficient intratubular cell clusters develop into frank tumors by growing into adjacent kidney tissue
- The nature of the “third hit” that causes frank tumor development remains unknown.

Percutaneous ablation of renal tumors in von Hippel-Lindau disease
Jean-Michel Correas, MD; Service de Radiologie; Hôpital Necker; Paris, France

Thermal ablation is a technique where either high heat or extreme cold is applied in a focused manner to tumors in the body. This approach is being used on kidney tumors in patients with VHL. At Hôpital Necker, 217 ablative procedures were performed on 67 patients with VHL (ages 18 – 87 years) were performed. Renal function tests indicated the start of loss of renal function in all patients. Renal function improved following percutaneous (needle puncture through the skin) ablation. The ablation area covered the tumor and there was some shrinking following ablation. No biopsies were done.

Conclusions:
Percutaneous ablations of renal tumors are:
- Safe and efficient
- Minimally invasive
- Cost effective
- Eliminate the need for general anesthesia
- Preserve renal parenchyma (functional cells) and kidney function
- Result in a long term success rate of over 95%
- Easy to repeat in the case of recurrence