VHL Research

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UT MD Anderson Cancer Center
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
- Generate Real-World Patient Databases

Therapeutic Avenues
VHL Gene and Protein

- On chromosome 3p25
- 213 amino acid protein
- Binds to Elongin C/B
- Forms “VBC complex”

Modified from Stebbins and Pavletich, Science, Vol 284, 16 April 1999
VHL - A Regulatory Hub

Regulates how the cell sees its surroundings

Regulates p53

Controls the primary cilium

Impacts blood vessel formation

Ohh et al, Mol Cell, Vol 1, 959-968, 1998
Kurban et al, Cancer Res 2006; 66: (3).

Roe and Youn Mol Cell May 2006

Thoma et al Nature Cell Biology Aug 2009
Kuehn et al Ca Res May 15, 2007

Pugh et al Nature Medicine 2003
Kerbel NEJM May 2008
VHL Mutation Replicates the Hypoxic State

Transcription of:
VEGF
Other angiogenic factors

VEGF = vascular endothelial growth factor; HIF = hypoxia-inducible factor.
In coming up with treatments we have to think about the different cells that make up the tumors.
Currently Evolving Treatment Paradigms

- **Targeting HIF**
  - HIF, HAF, VEGF Modulating Agents and Metabolism Modifiers

- **Restabilizing and refunctionalizing mutated VHL**
  - Modulators of VHL Proteostasis

- **Targeting the tumor cell**
  - Modulators of Autophagy, or of co-Mutated Genes

- **Targeting the immune microenvironment**
  - Immune Checkpoint Inhibitors
**Over One Third of Mutations are Missense (Hereditary and Sporadic)**


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**TABLE 2. Types of Mutation Found in von Hippel-Lindau Disease Without (Type 1) and With (Type 2) Pheochromocytoma**

<table>
<thead>
<tr>
<th>VHL type</th>
<th>Number of families</th>
<th>Missense</th>
<th>Nonsense</th>
<th>Micro deletion (1–9 bp)</th>
<th>Insertion (1–8 bp)</th>
<th>Deletion (4–380/kb)</th>
<th>Splice site</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>336</td>
<td>72</td>
<td>29</td>
<td>31</td>
<td>13</td>
<td>53</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>78</td>
<td>61</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>414</td>
<td>133</td>
<td>30</td>
<td>31</td>
<td>15</td>
<td>55</td>
<td>10</td>
</tr>
</tbody>
</table>

*Each type of mutation was tested by Fishers exact test (2-tailed) for association with VHL types 1 or 2. Microdeletions/insertions (P = 0.019), nonsense (P = 0.044), and deletion mutations (P 0.012) were predictive of VHL type 1. Missense mutations were significantly more common in VHL type 2 (P = <0.001). Fifty-three VHL families without information sufficient to classify into VHL1, VHL2 were excluded from this analyses.*

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What this means is you have a full sized protein, that can possibly be fixed
Mutated VHL Has a Shorter Lifespan in the Cell Due To Accelerated Degradation

A) VHL WT-Venus

B) VHL(W117A)-Venus

Jonasch Lab
Mutated VHL Has a Shorter Lifespan in the Cell Due To Accelerated Degradation

Do some mutated VHL subtypes maintain residual functionality?
Mutated VHL Has a Shorter Lifespan in the Cell Due To Accelerated Degradation

Can we rescue these subtypes using genetic and pharmacological means?
Point Mutations Destabilize VHL But May Retain Functionality

C-terminal Venus Tagged Proteins

Jonasch Lab
R167Q mutation found in the elongin C binding region of VHL, and prevents VBC complex formation.

Is most common VHL mutation in VHL disease patients.
VHL Mutational Isoforms Influence Renal Cell Carcinoma Growth

*Isofluorane Anesthesia, 1 x 10^7/100µl PBS, sc into both flanks, 21G, per cell line.

*Treatment to begin 2 weeks post tumor implantation.

Group A: 786-0 parental line

Group B: 786-0 with L117A mutation and a C-terminal Venus tag

Group C: 786-0 with R167Q mutation and a C-terminal Venus tag

Group D: 786-0 with R167Q mutation and no tag

Ding and Jonasch Ca Research 2014
Bortezomib raises VHL levels and lowers HIF and GLUT1 levels

Ding and Jonasch Ca Research 2014
A novel chemical chaperone for treating the VHL cancer syndrome

Danny Segal
Dept. Molecular Microbiology & Biotechnology
Tel Aviv University
Arginine

An amino acid, used as a building block to make proteins

You can get left-handed and right-handed versions
Dr. Segal’s lab indicates that using both D- and L-arginine may normalize HIF regulation of various mutant VHL isoforms.
Ongoing work will further refine the list of candidate molecules capable of refunctionalizing and restabilizing VHL.
2014 Full Project Awardee

VHL Models and Novel Therapeutics

Othon Iliopoulos
Dept. Oncology
Massachusetts General Hospital, Boston MA
• Zebrafish are tiny fish that can be genetically modified.
• VHL mutation in zebrafish can represent aspects of human biology.
• Dr. Iliopoulos will use zebrafish to discover new drugs that may rescue consequences of VHL mutation.
• Work is underway and will be finalized next year.
Salivary, plasma metanephrines and anxiety levels in pheochromocytomas (STRESS)

A.N.A van der Horst-Schrivers
Department of Endocrinology
University Medical Center Groningen

2015 Pilot Project Awardee
Rationale

- Measurement of metabolites of catecholamines (metanephrines) is the cornerstone in diagnosing a pheochromocytoma.
- Carriers of germline mutations such as VHL are annually screened for a pheochromocytoma using blood to measure metanephrines.
- However, for this test rest for 30 minutes in supine position before blood sampling is obligatory.
- Measurement of metanephrines in saliva could be less cumbersome, and more patient friendly. It has the advantage of collection at home (and subsequently send by mail to the hospital).
Approach

• This study aims to determine whether the saliva test is just as accurate and sensitive as the measurement of metanephrines in blood.
• This study will be performed in the Netherlands and at the National Institute of Health (NIH), Bethesda, USA.
• Investigators will include 145 patients with a PCC, 145 healthy controls and 145 germline mutation carriers.
Significance

• If measurement of salivary metanephrines is just as accurate as blood metanephrines, then this approach will be more time and cost effective for patients/germline mutation carriers and for the treating medical team.
Using a novel mouse model of ccRCC to investigate Hif-1α and Hif-2α inhibition for cancer prevention and therapy

Prof. Dr. Ian J. Frew
Institute of Physiology, University of Zurich
Rationale

• Clear cell renal cell carcinomas (ccRCC) are kidney tumours that arise very frequently in patients with the inherited von Hippel-Lindau (VHL) disease syndrome.

• The generation of mouse models of human tumours using genetically modified mice has been a powerful tool used by scientists to not only understand the genetic causes and biological behaviour of tumours but also to test new therapies that can guide subsequent drug trials in human patients.
Approach

• Dr. Frew and his team have recently generated a very good mouse model of ccRCC, possibly the first that truly represents what happens in patients.

• They will use mouse ccRCC model to determine whether drug treatment can prevent the formation of new tumors and efficiently treat existing tumors. They will test available compounds that block HIF.
Significance

• The information gained from this combination of a genetic and a pharmacological approach will be highly useful to guide new trials in individuals with VHL disease and in patients with noninherited clear cell renal cell carcinoma.
Cancer in Our Genes International Patient (CGIP) Databank

A patient-driven databank dedicated to finding a cure for VHL, BHD, HLRCC, SDH, and related disorders
• Outcome of 10th International VHL Medical Symposium (Houston, 2012)
  – VHLA Research Council

• Collaborative effort includes National Organization of Rare Disorders (NORD)
  – NORD = Software Provider
  – VHLA = Databank Owner
CGIP: A Complementary Effort

• Joint effort between VHLA and health care professionals

• Complementary to existing institutional databanks
  – Information best answered by patients, i.e. Lifestyle (diet, exercise, sleep, nutritional supplements, mood, altitude, oral health)

• De-identified data available to researchers

• Match participants within a specific research criteria

• Provide baseline data for clinical trial
CGIP Goals

• Further understand natural history
  - Longitudinal

• International study
  - Wide range of genotype
  - Study geographical differences

• Comprehensive patient-driven data
  - Impact of lifestyle on disease progression and/or tumor growth rate

• Learn from all experimentation

• Learn from commonalities and differences between disorders
CGIP Features

- Privacy and Confidentiality: Primary concerns and factor built into CGIP
- Confidential/Secure
- IRB Approved
- Data curation process incorporated
- Online: no geographic limitations
- Language = English
- No age limitations
CGIP Surveys

- About the Participant
- Diagnosis and Medical History
- Genetics
- Eye
- Ear
- Kidney
- Neurology
- Pancreas and Digestive Issues
- Adrenal

- Heart
- Reproductive Tract
- Thyroid
- Lung
- Skin
- Nutrition and Exercise
- Oral Health and Tobacco Use
- Measuring your Mood
- Other Information and Updates
CGIP Status

- Launched March 2014
- "Living Registry": Updates based on learnings
- Registrants vs. Participants
  502 vs. 344 or 68.5%
## CGIP Demographics

### Gender

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>Female</td>
<td>66 %</td>
</tr>
<tr>
<td>Male</td>
<td>34 %</td>
</tr>
</tbody>
</table>

### Age

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>Median</td>
<td>43 yrs</td>
</tr>
<tr>
<td>Min</td>
<td>13 yrs</td>
</tr>
<tr>
<td>Max</td>
<td>81 yrs</td>
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</tbody>
</table>

### Country of Residence

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>US</td>
<td>76 %</td>
</tr>
<tr>
<td>EU</td>
<td>11 %</td>
</tr>
<tr>
<td>Canada</td>
<td>6 %</td>
</tr>
<tr>
<td>Pacific/Asia</td>
<td>4 %</td>
</tr>
<tr>
<td>South America</td>
<td>2 %</td>
</tr>
<tr>
<td>Other</td>
<td>1 %</td>
</tr>
</tbody>
</table>

### Diagnosis (self reported)

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>VHL</td>
<td>84 %</td>
</tr>
<tr>
<td>HLRCC</td>
<td>7 %</td>
</tr>
<tr>
<td>BDH</td>
<td>6 %</td>
</tr>
<tr>
<td>SDH</td>
<td>2 %</td>
</tr>
<tr>
<td>Other/Undiagnosed</td>
<td>1 %</td>
</tr>
</tbody>
</table>
## CGIP Data

### General Health (self-reported)

<table>
<thead>
<tr>
<th>Health Level</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excellent</td>
<td>12%</td>
</tr>
<tr>
<td>Very Good</td>
<td>31%</td>
</tr>
<tr>
<td>Good</td>
<td>31%</td>
</tr>
<tr>
<td>Fair</td>
<td>19%</td>
</tr>
<tr>
<td>Poor</td>
<td>6%</td>
</tr>
</tbody>
</table>

### Alcohol Consumption

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daily</td>
<td>6%</td>
</tr>
<tr>
<td>4-6 times/wk</td>
<td>7%</td>
</tr>
<tr>
<td>2-3 times/wk</td>
<td>14%</td>
</tr>
<tr>
<td>1 times/wk</td>
<td>15%</td>
</tr>
<tr>
<td>Rarely/Not at all</td>
<td>57%</td>
</tr>
</tbody>
</table>

### BMI

<table>
<thead>
<tr>
<th>BMI Category</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Underweight: &lt; 18.5</td>
<td>3%</td>
</tr>
<tr>
<td>Normal weight: 18.5 - 24.9</td>
<td>36%</td>
</tr>
<tr>
<td>Overweight: 25.0 - 29.9</td>
<td>33%</td>
</tr>
<tr>
<td>Obese: &gt; 30</td>
<td>29%</td>
</tr>
</tbody>
</table>
CGIP Data

- Does your health now limit you in doing **vigorous activities**?

- How often do you feel **fatigued**?

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Never/Rarely</td>
<td>25%</td>
</tr>
<tr>
<td>Sometimes</td>
<td>33%</td>
</tr>
<tr>
<td>Often/Always</td>
<td>42%</td>
</tr>
</tbody>
</table>

- How frequently do you do at least **10 minutes of sustained exercise** in a day? (walking, yoga, weight training)

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daily</td>
<td>28%</td>
</tr>
<tr>
<td>4-6 times/wk</td>
<td>22%</td>
</tr>
<tr>
<td>2-3 times/wk</td>
<td>24%</td>
</tr>
<tr>
<td>1 time/wk</td>
<td>9%</td>
</tr>
<tr>
<td>Rarely</td>
<td>12%</td>
</tr>
<tr>
<td>Not at all</td>
<td>5%</td>
</tr>
</tbody>
</table>
**CGIP Data**

- **How frequently do you eat at least 1 cup of fruit or fruit juice or 1/2 cup of fresh or frozen vegetables?**

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Fruit/Juice</th>
<th>Vegetables</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daily</td>
<td>43%</td>
<td>47%</td>
</tr>
<tr>
<td>4-6 times/wk</td>
<td>23%</td>
<td>25%</td>
</tr>
<tr>
<td>2-3 times/wk</td>
<td>18%</td>
<td>22%</td>
</tr>
<tr>
<td>1 time/wk</td>
<td>6%</td>
<td>3%</td>
</tr>
<tr>
<td>Rarely/Not at all</td>
<td>10%</td>
<td>7%</td>
</tr>
</tbody>
</table>
CGIP Preliminary Data

Oral Health out of 167 patients

• Dry Mouth = 29.5%
  (normal for age 70+)

• Mouth Sores (aphtha) = 47.9%
  (very high digestive issues?)

• Root Canal (one or more) = 39.5%
  (high, generally 20%)

• Crowns (one or more) = 43.7%
  (consistent with high root canal)
CGIP Challenges

- Global support and participation by researchers
- Increased awareness among patients
  - VHL, BHD, HLRCC, SDH, etc.
- Increasing participation
- Patient follow-through
  - Surveys
  - Medical information
Past Present and Future

Identification of the VHL Gene

Determining *how* VHL deficiency affect patients

Developing new ways to treat VHL disease