Basic and clinical research in pheochromocytoma (PHEO): a winning combination

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Disclosure

Nothing to disclose

Lecture outline

The lecture will focus on the main discoveries over the course of the past 4 years.

1. Summarize current basic-clinical discoveries that represent a winning combination in how we diagnose PHEO patients today:
   a/ genetics
   b/ biochemistry
   c/ metabolomics
   d/ functional imaging

2. Promising future therapeutic approaches derived from basic research discoveries

3. Conclusions/perspectives

PHEO/PGL: Continuing progress

Adapted from G. Eisenhofer

PGL: paraganglioma
PHD1: prolyl hydroxylase 1
MDH2: malate dehydrogenase 2

NF1: neurofibromatosis type 1
VHL: von Hippel-Lindau
RET: rearranged during transfection
SDH: succinate dehydrogenase
TMEM127: transmembrane protein 127
HIF2A: hypoxia inducible factor 2 alpha
PHD2: prolyl hydroxylase domain-containing protein 2
IDH1: isocitrate dehydrogenase 1
H-RAS: Harvey-Ras protein
FH: familial hypercholesterolemia

Colorimetric Assays
HPLC Assays
LC-MS/MS (routine)
PET Ligands
DOTA analogs

Improved understanding of catecholamine metabolism
Shift from catecholamines to metanephrines
Methoxytyramine

**PHEO/PGL: Facts**

**Current important facts:**

- In over 50% of PHEOs/PGLs, a genetic defect is known (35-40% have germline and 15-25% harbor somatic mutations); 19 PHEO/PGL susceptibility genes are currently known.
- Biochemical dx. and localization are highly successful.
- There is no satisfactory cure for metastatic PHEO/PGL.
- International studies and consortia are evolving.

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**New genes: A story of metabolic/oxidative stress and hypoxia continues**

- Proteomics & metabolomics: The way to new discoveries
  - Improve understanding of PHEO/PGL cell biology
  - Improve clinical care for afflicted patients

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**Proteins & metabolomics: The way to new discoveries**

- Enzymes: fumarate hydratase (FDH), malate dehydrogenase (MDH2), HIF2A, PHD1
- Functions: DNA instability, cell growth, proliferation, angiogenesis, myeloproliferative disease, T-cell proliferation, cell migration, invasion, and survival

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**Improved understanding of PHEO/PGL cell biology**

- Metabolomics, Proteomics: The way to new discoveries
We have found that about 70% of SDHB/D PHEOs and 20-30% of head and neck PGLs secrete MTY.

Methoxytyramine (MTY), tumor size, extra-adrenal location, and SDHB as independent predictors of metastatic PHEO/PGL

365 patients with PHEO, including 105 with metastases, 846 subjects without tumors

MTY & METASTASIS
4.7-fold higher in patients with than without metastases; independent of tumor burden and NMN levels

SIZE & LOCATION

156 tumors included for genotype-specific catecholamine profiling

Strategy for new genetic screening

- There is a need for a more cost-effective approach
- Immunostaining approaches are less expensive than genetic screening

SDHB immunohistochemistry detects patients with SDHx-related PHEO/PGL

Immunohistochemistry can be used to triage genetic testing; also to detect recently described a SDHA gene mutation in PHEO/PGL
Lendvai et al. Endocrinology, 2014; 155:27

PHEO/PGL & metabolomics (1)

- 234 PHEOs/PGLs from 233 pts (45 had SDHx gene mutations).
- Training set: 50 tumors; validation set: 184 tumors.
- Metabolite extraction from fresh-frozen tumors, HPLC-tandem MS used.

Succinate:fumarate ratio

<table>
<thead>
<tr>
<th>SDHB/D (n=11)</th>
<th>non-SDH (n=39)</th>
<th>p&lt;0.001</th>
</tr>
</thead>
<tbody>
<tr>
<td>Succinate to fumarate ratio as a predictor for SDHx mutations</td>
<td></td>
<td></td>
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</table>

Trapping set Validation set

<table>
<thead>
<tr>
<th>SDHB/C/D (n=32)</th>
<th>non-SDH (n=150)</th>
<th>p&lt;0.001</th>
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</table>

Training set Validation set

Table 1. Diagnostic sensitivity and specificity for succinate:fumarate ratios to identify SDHx mutations

<table>
<thead>
<tr>
<th>All PPGLs</th>
<th>PPGLs excl. HNP</th>
<th>HNP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity [%]</td>
<td>90.9 (30/33)</td>
<td>100 (15/15)</td>
</tr>
<tr>
<td>Specificity [%]</td>
<td>97.3 (145/149)</td>
<td>97.9 (141/144)</td>
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Training and validation set

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<td>Sensitivity [%]</td>
<td>93.2 (41/44)</td>
<td>100 (25/25)</td>
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<tr>
<td>Specificity [%]</td>
<td>96.8 (181/187)</td>
<td>97.3 (177/182)</td>
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HNP- head and neck paraganglioma

HIGH diagnostic performance

Krebs cycle changes beyond succinate and fumarate

MAX: Tumor suppressor gene

- MYC-associated factor X regulates cell proliferation, apoptosis, differentiation; dimerization of MYC-MAX
- PHEO, bilateral; rarely PGL
- Malignancy in about 10-15% of patients
- Paternal transmission (similar to SDHD, SDHAF2)
- Produce NE + EPI


Metabolomics to predict the pathogenicity of unknown SDHx mutations

IHC: SDHB

FDOPA

HRMAS

Modelling

Taieb et al. personal communication

Richter et al. JCEM, 2014; 99:3903

PHEO/PGL & metabolomics (2)
Opposing effects of HIF-1α and HIF-2α on chromaffin cell phenotypic features: Insights from MYC-associated factor X (MAX)

- MAX plays an important role in the control of the MYC/MAX pathway and contributes to numerous neoplastic conditions, including neuroblastoma.
- Recently, MAX mutations have been found in some PHEOs/PGLs.
- HIF-2α enhances MYC interaction with MAX to stimulate cancer cell progression; HIF-1α has the opposite effect.

The new syndrome of PGL, somatostatinoma and polycythemia

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<th>Patient 1</th>
<th>Patient 2</th>
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<td>At birth</td>
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<td>NA</td>
</tr>
<tr>
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<td>8 yr</td>
<td>At birth</td>
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</tr>
<tr>
<td><strong>Blue feet</strong></td>
<td>No</td>
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<td>NA</td>
</tr>
<tr>
<td><strong>Erythrocytes</strong></td>
<td>7,780,000</td>
<td>7,850,000</td>
<td>5,220,000</td>
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<td><strong>Hematocrit</strong></td>
<td>50.5</td>
<td>59.3</td>
<td>44.9</td>
</tr>
<tr>
<td><strong>Erythropoietin</strong></td>
<td>150</td>
<td>180</td>
<td>36.7</td>
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<td><strong>Multiple PGLs</strong></td>
<td>14 yr</td>
<td>18 yr</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Normetanephrine</strong></td>
<td>4,834</td>
<td>858</td>
<td>112</td>
</tr>
<tr>
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<td>29 yr</td>
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Zhuang et al. NEJM 2012; 367:922-930

Qin et al. J. Cancer 2014; 135:2054

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Previous studies related to PHEO/PGL and polycythemia served as a clue to discovering a new syndrome

Somatic gain-of-function HIF-2α (HIF2A) mutation

HIF-2α (HIF2A) mutation

Blood Tumor (PGL) Tumor (SOM)

Patient 1

Pt. 1. A530T

Patient 2

Pt. 2. A530V

Somatic HIF2A Gain-of-Function Mutations in Paraganglioma with Polycythemia

Zhuang et al. NEJM 2012; 367:922-930

Kaelin Nat. Rev. Cancer 2008; 8:865
Ladroue et al. NEJM 2008; 359:2685

HIF-2α IHC

N: normal adrenal medulla

Pt. 1. A530T

Pt. 2. A530V
Other genetic abnormalities in patients with HIF2A mutations

A gain of 2p chromosome

Oncogenic effects of HIF2A mutations in mice

Some patients without polycythemia: related to the time at which the mutation occurs?

HIF2A mutations: more patients are being discovered

<table>
<thead>
<tr>
<th>Age of onset</th>
<th>Mutation</th>
<th>Amino Acid</th>
<th>Somatic mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>C1591T</td>
<td>P531S</td>
<td>Y</td>
</tr>
<tr>
<td>25</td>
<td>C1590T</td>
<td>A530V</td>
<td>Y</td>
</tr>
<tr>
<td>18</td>
<td>G1588A</td>
<td>A530T</td>
<td>Y</td>
</tr>
<tr>
<td>13</td>
<td>C1591T</td>
<td>P531S</td>
<td>Y</td>
</tr>
<tr>
<td>18</td>
<td>C1592T</td>
<td>P531L</td>
<td>Y</td>
</tr>
<tr>
<td>43</td>
<td>C1600_1608del</td>
<td>P534_D536del</td>
<td>Y</td>
</tr>
<tr>
<td>78</td>
<td>G1615T</td>
<td>D539Y</td>
<td>Y</td>
</tr>
<tr>
<td>10</td>
<td>C1591A</td>
<td>P531T</td>
<td>Y</td>
</tr>
<tr>
<td>47</td>
<td>C1591T</td>
<td>P531S</td>
<td>Y</td>
</tr>
<tr>
<td>72</td>
<td>C1592T</td>
<td>P531L</td>
<td>Y</td>
</tr>
<tr>
<td>7</td>
<td>C212A</td>
<td>S71Y</td>
<td>Y</td>
</tr>
<tr>
<td>16</td>
<td>T1586C</td>
<td>L529P</td>
<td>Y</td>
</tr>
<tr>
<td>2</td>
<td>C1625T</td>
<td>L542P</td>
<td>Y/N</td>
</tr>
<tr>
<td>15</td>
<td>C1589A</td>
<td>A530E</td>
<td>Y</td>
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New germline PHD1 (EGLN2) mutation

• Some patients with polycythemia and PHEO do not have the HIF2A mutation and they present with normal or slightly elevated EPO levels
• Germline PHD2 mutations described previously in patients with polycythemia and in one patient also with PGL

SDHx: A hypermethylator phenotype

145 PHEOs/PGLs were included (17 SDHx, 21 VHL, 30 NF1, 13 RET and 64 others).

Immortalized mouse chromaffin cells harboring a complete defect in SDH
• Increased migration, well inhibited by decitabine (inhibits DNA-methyltransferase activity)
• Histone methylation was increased (also in human samples)
PHEO and somatostatin receptors: imaging

- PHEOs express 5 somatostatin receptors (SSTRs), allowing for the use of Octreoscan scintigraphy (relatively poor spatial resolution)
- SSTR imaging can be performed with PET/CT, improving spatial resolution; also provides more rapid and whole-body tomographic imaging for precise anatomic localization
- Available 3 DOTA-coupled peptides include: $^{68}$Ga-DOTATATE, $^{68}$Ga-DOTATOC, $^{68}$Ga-DOTANOC

Future treatment options: HIF-α inhibitors

SDHB missense mutations: It is about protein degradation, not production
Other future promising targets for the treatment of metastatic PHEO/PGL

**Future therapeutic targets**

1. Mitochondrial proton pump modulators
2. SDHB stabilization (HDAC inhibitors)
3. Inhibition of cholesterol synthesis
4. Topoisomerase inhibitors
5. Demethylating agents (5-Azacytidine)

CONCLUSIONS/PERSPECTIVES

- Further promote tight collaboration, information exchange, and unique teamwork between patients, clinicians, and scientists across various institutions.

  Together we have a chance; separately we fail…

Acknowledgements

Many thanks to all the members of my laboratory, attendings, and endocrine, oncology, and surgery fellows for their long hours, dedication, and passion for helping those who suffer.

Many thanks to outside NIH co-investigators:

"Patients are our passion and we are their hope"
HIF-2α signaling in cluster 1 hereditary PHEO/PGL: Turning the rudder in the right direction?

HIF-1/2α signaling in cluster 2 hereditary PHEO/PGL: Turning the rudder in the right direction?

The Cancer Genome Atlas

Understanding genomics to improve cancer care

TCGA facts:
1. A comprehensive, collaborative effort led by the NIH
2. Announced in 2009 to map the genomes of at least 20 cancers by 2014
3. Nine rare cancers included – PHEO/PGL is one of them (2012)
4. Data types: whole exome, mRNA, and miRNA sequencing, DNA methylation, and DNA copy number
5. Researchers across the US and other nations to collaborate
6. The PHEO/PGL project is expected to be completed by 2015

The glory of medicine is that it is constantly moving forward, that there is always more to learn.

Dr. William J. Mayo, 1928