Hypoxia-inducible factors in erythropoiesis, iron homeostasis and beyond

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@HIFpathway
Outline: HIF in erythropoiesis

- overview
- EPO synthesis in kidney and liver
- iron metabolism
- renal anemia
- HIF beyond erythropoiesis
Physiologic Responses to Hypoxia:
This happens when you go to high altitude

Andrew M. Luks J Appl Physiol 2015;118:509-519

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EPO and Hgb responses at high altitude

Faura et al., Blood, 1969

Abbrecht and Littel, J. Appl. Physiol., 1972
HIF controls the Hypoxic Induction of EPO

Hypoxia → HIF → EPO → sEPO → Erythropoiesis

Haase, VH
HIF regulates multiple processes: biological target complexity

**Metabolism:**
- Glycolysis (HIF-1)
- Fat metabolism (HIF-2)
- Adenylate kinase-3
- Carbonic anhydrase-9
- Glut-1 and -3
- Glycolytic enzymes (Hexokinase, LDH, PGK, etc.)
- Leptin

**Iron metabolism/Erythropoiesis**
- Ceruloplasmin
- EPO (HIF-2 > HIF-1)
- Transferrin
- Transferrin R

**Epithelial Barrier Function**
- ITF, MDR-1, CD73

**ECM production/Cell migration**
- CxCR4, c-met, CTGF, PAI-1, Procollagen prolyl hydroxylase-α1

**Proliferation/Cell survival**
- Cyclin G2, EPO
- Heme oxygenase 1
- IGF-2, IGFBP1, -2, -3
- NOS-2, NIP-3, p21
- TGF-β3, VEGF, WT1
- Indirect: c-Myc

**Vasculogenesis/angiogenesis**
- Heme oxygenase-2
- NOS-2, PAI-1
- VEGF, VEGF-R (FLT-1)

**Transcription factors**
- Ets-1, DEC-1/Stra13

**HYPOXIA via HIF**

Haase, VH
Regulation of HIF-α stability: molecular complexity

HIF: hypoxia-inducible factor

Haase, VH, AJP Renal 2006
Discovery of HIF-oxygen sensing


2OG-dependent dioxygenases: pharmacologic target complexity

PHD1, PHD2, PHD3, FIH

Loenarz and Schofield, Nature Chemical Biology, 2008
The HIF-EPO axis in kidney and liver
HIF-2 dependent regulation of EPO

EPO: erythropoietin; HIF: hypoxia-inducible factor.
EPO-producing cells in human kidneys

HIF-2 regulates the size of the REPC pool

Distinct interstitial cell populations regulate renal EPO production: cellular complexity

EPO: erythropoietin; PHD: prolyl-4-hydroxylase domain; REPC: renal EPO-producing cell.
The liver as EPO source: role of individual PHDs


EPO: erythropoietin; PHD: prolyl-4-hydroxylase domain.

ControlP13-L-KO
P23-L-KO
P12-L-KO
P13-L-KO
P23-L-KO
P123-L-KO

KDIGO
HIF and iron metabolism
HIF in iron metabolism

HIF-2 iron feedback via IRP

Fe-S Cluster Biogenesis
Iron Deficiency Phosphorylation

IRE
Hif-2α mRNA
Translational Activation

IRP1

Renal Epo mRNA
Serum EPO (pg/mL)

control
Irp1Δ

control
Irp1Δ

Epo
Pdgfβ

Epo+ cells/mm²

rbc 10⁹/ml

0.5 7 21 28 35 56 119 (d)
Key Points

- The hypoxic induction of EPO in the kidney and liver is HIF-2-dependent.
- Inactivation of PHD2 alone is sufficient to stimulate the production of renal EPO.
- There are at least two distinct populations of EPO-producing cells in the kidney that differ in their regulation of HIF-2 activity and EPO production.
- Inactivation of at least 2 PHD enzymes in the liver is required to stimulate erythropoiesis.
- HIF coordinates erythropoiesis with iron metabolism.
- Intracellular iron regulates HIF oxygen sensing.

EPO: erythropoietin; HIF: hypoxia-inducible factor; PHD: prolyl-4-hydroxylase domain.
HIF-PHD Inhibitors for renal anemia therapy:
overview of compounds and mechanism
Pathogenesis of Renal Anemia

EPO: erythropoietin.
HIF-PHD as pharmacologic target

\[ \text{HIF-α} \]

2OG oxygenases

HIF-PHDs (PHD1, PHD2, PHD3)
HIF-PHI
chemical structures

2-Oxoglutarate (2OG)
MW: 146.1 g/mol

Dimethylxalylglycine (DMOG)
MW: 175.1 g/mol

FG-2216
MW: 280.7 g/mol

FG-4592 / Roxadustat
MW: 352.3 g/mol

GSK-1278863 / Daprodustat
MW: 393.4 g/mol

BAY-85-3934 / Molidustat
MW: 314.3 g/mol

AKB-6548 / Vadadustat
MW: 306.9 g/mol
Pharmacologic profiles of HIF-PHIs

<table>
<thead>
<tr>
<th>Compound</th>
<th>Effective daily oral doses in phase II trials</th>
<th>Dosing Schedule</th>
<th>half-life</th>
<th>Plasma EPO (IU/L)</th>
<th>Metabolism</th>
<th>rel. activity KM (µm)</th>
<th>KDIGO</th>
</tr>
</thead>
<tbody>
<tr>
<td>daprodustat</td>
<td>5-25 mg (also examined 50 and 100 mg)</td>
<td>QD</td>
<td>~1-7 hrs</td>
<td>24.7 and 34.4, 82.4 b</td>
<td>CYP2C8 with minor CYP3A4</td>
<td>PHD3&gt;PHD1&gt;PHD2</td>
<td></td>
</tr>
<tr>
<td>(GSK-12278863)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.007</td>
<td></td>
</tr>
<tr>
<td>molidustat</td>
<td>25-150 mg (&gt;75mg in DD-CKD)</td>
<td>QD</td>
<td>4-10 hrs</td>
<td>39.8 d</td>
<td>n.r.</td>
<td>0.007</td>
<td></td>
</tr>
<tr>
<td>(BAY 85-3934)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>PHD3&gt;PHD1/PHD2</td>
<td></td>
</tr>
<tr>
<td>roxadustat</td>
<td>0.7-2.5 mg/kg</td>
<td>TIW</td>
<td>12-15 hrs</td>
<td>113 and 397, 130 f</td>
<td>CYP2C8</td>
<td>PHD1,2,3</td>
<td>0.027</td>
</tr>
<tr>
<td>(FG-4592, ASP1517)</td>
<td></td>
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<tr>
<td>vadadustat</td>
<td>150-600 mg</td>
<td>QD (TIW)</td>
<td>4.7-9.1 hrs</td>
<td>32 h</td>
<td>n.r.</td>
<td>PHD3&gt;PHD1&gt;PHD2</td>
<td></td>
</tr>
<tr>
<td>(AKB-6548, MT-6548)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.029</td>
<td></td>
</tr>
</tbody>
</table>

Others:
- **Enarodustat** (JTZ-951, Japan Tabacco),
- **Desidustat** (Zyann1, Cadila Healthcare/Zydus Cadila),
- **TP0463518** (Taisho Pharmaceutical)

Sanghani and Haase, ACKD, 2019
HIF-PHI: mechanisms in renal anemia
Renal EPO-producing cells in CKD

CKD: chronic kidney disease; EPO: erythropoietin; REPC: renal EPO-producing cell.

HIF-Prolyl Hydroxylase Inhibition: potential adverse effects

- VEGF?
- Metabolic effects (glucose, cholesterol, fat metabolism, uric acid, FGF 23) ?
- Pulmonary artery pressure ?
- Systemic arterial blood pressure ?
- Effects on kidney disease progression ?
- Liver toxicity ?
- Pro-oncogenic potential ?
HIF activation:
potential applications in renal injury
## Preclinical studies in AKI – evidence for renoprotection

<table>
<thead>
<tr>
<th>Reference</th>
<th>Approach</th>
<th>Cell type</th>
<th>Dose and time</th>
<th>Clinical outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Matsumoto 2003</td>
<td>CoCl₂</td>
<td>systemic</td>
<td>2 mM CoCl₂ in drinking water, day −10, right nephrectomy and left renal IRI</td>
<td>reduction of tubulointerstitial damage, vimentin expression, inflammation, increase in Ho1, Glut1, and Vegf mRNA levels in rat kidneys 3 d post-op</td>
</tr>
<tr>
<td>Bernhardt 2006</td>
<td>0.1% CO</td>
<td>epithelial</td>
<td>CO exposure for 10 h prior to right-sided nephrectomy and left renal IRI</td>
<td>72 h after IRI, left kidneys from pretreated vs control rats had reduced tubular necrosis and apoptosis, and less macrophage infiltration</td>
</tr>
<tr>
<td>Bernhardt 2006</td>
<td>FG-4487</td>
<td>epithelial</td>
<td>Single dose (25 mg/kg i.p.) 6 h before right-sided nephrectomy and left renal IRI</td>
<td>72 h after ischemia, left kidneys from pretreated vs control rats had reduced tubular necrosis and apoptosis</td>
</tr>
<tr>
<td>Hill 2008</td>
<td>DMOG + global HIF KO</td>
<td>systemic</td>
<td>3 doses 8 mg i.p., 48 h and 6 h before IRI and 48 h after IRI</td>
<td>72 h after IRI, DMOG-treated mice had less tissue damage, apoptosis, and macrophage infiltration vs controls</td>
</tr>
<tr>
<td>Schley 2011</td>
<td>conditional KO of VHL in TAL</td>
<td>epithelial (TAL)</td>
<td>genetic model</td>
<td>Attenuation of proximal tubular injury, preservation of TAL function</td>
</tr>
<tr>
<td>Kapitsinou 2012</td>
<td>GSK1002083A</td>
<td>systemic</td>
<td>2 oral doses of GSK1002083A at 48 h and 6 h before renal IRI, or days 2 and 4 post IRI</td>
<td>GSK1002083A pretreatment (but not posttreatment) preserved kidney function and prevented fibrosis, inflammation, and anemia in mice 21 d post IRI</td>
</tr>
<tr>
<td>Fähling 2013</td>
<td>inducible KO of VHL</td>
<td>pan-epithelial</td>
<td>tetracycline-inducible genetic model</td>
<td>HIF-mediated renoprotection in VHL KO mice via metabolic shift toward glycolysis in tubules at day 1 after rhabdomyolysis</td>
</tr>
<tr>
<td>Kapitsinou 2014</td>
<td>GSK1002083A</td>
<td>endothelial</td>
<td>genetic model</td>
<td>3 oral doses: 2 d before IRI, 6 h before IRI, 2 d after IRI</td>
</tr>
<tr>
<td>Yang 2018</td>
<td>FG-4592 (Roxadustat)</td>
<td>epithelial</td>
<td>10 mg/kg/day i.p., 48 h prior to cisplatin-induced AKI</td>
<td>GSK1002083A attenuated renal injury at day 3 after IRI in wild-type mice but not in mice with inactivation of endothelial HIF-2α</td>
</tr>
</tbody>
</table>
Potential mechanisms of HIF-dependent
“Ischemic preconditioning”

Kapitsinou and Haase., AJP Renal, 2015
Key Points

- There is strong preclinical evidence for HIF-induced protection from acute ischemic injury warranting further investigation in patients.

- HIF protects from renal ischemia-reperfusion injury and transition to CKD.

- Are currently used dosing regimen sufficient to afford cytoprotection (Dapro trial in PVD failed)?

- The effects of HIF activation on chronic kidney injury are controversial. Preclinical studies indicate strong cell type and context dependence.
HIF oxygen sensing
cardiovascular disease

KDIGO
Cardiovascular risk in CKD: is HIF-PHI therapy beneficial?

Gansevoort RT et al., Lancet, 2013
Altitude reduces all-cause mortality in incident dialysis

Table 2. Unadjusted and Adjusted Relative Mortality in US Patients Receiving Dialysis

<table>
<thead>
<tr>
<th>Residential Elevation, m</th>
<th>&lt;76</th>
<th>76-609</th>
<th>610-1218</th>
<th>1219-1828</th>
<th>&gt;1828</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of deaths</td>
<td>177,412</td>
<td>238,214</td>
<td>12,046</td>
<td>7,380</td>
<td>1,720</td>
</tr>
<tr>
<td>Mortality rate (95% CI)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Per 1000 person-years</td>
<td>220.1 (219.1-221.2)</td>
<td>221.2 (220.3-222.1)</td>
<td>214.6 (210.3-218.5)</td>
<td>184.9 (180.7-189.1)</td>
<td>177.2 (169.0-185.7)</td>
</tr>
<tr>
<td>Unadjusted</td>
<td>1.0 [Reference]</td>
<td>1.00 (1.00-1.01)</td>
<td>0.97 (0.96-0.99)</td>
<td>0.85 (0.83-0.87)</td>
<td>0.81 (0.78-0.85)</td>
</tr>
<tr>
<td>Adjusted for age, sex, race, Medicaid coverage</td>
<td>1.0 [Reference]</td>
<td>0.99 (0.98-1.00)</td>
<td>0.95 (0.93-0.97)</td>
<td>0.85 (0.83-0.87)</td>
<td>0.83 (0.79-0.87)</td>
</tr>
<tr>
<td>Additionally adjusted for comorbidities, inability to ambulate, inability to transfer, baseline rEPO use, and dialysis modality</td>
<td>1.0 [Reference]</td>
<td>0.97 (0.97-0.98)</td>
<td>0.96 (0.94-0.97)</td>
<td>0.86 (0.84-0.88)</td>
<td>0.85 (0.81-0.89)</td>
</tr>
<tr>
<td>Additionally adjusted for BMI, estimated GFR, hemoglobin, and serum albumin</td>
<td>1.0 [Reference]</td>
<td>0.97 (0.96-0.98)</td>
<td>0.93 (0.91-0.95)</td>
<td>0.88 (0.84-0.91)</td>
<td>0.85 (0.79-0.92)</td>
</tr>
</tbody>
</table>

Abbreviations: BMI, body mass index; CI, confidence interval; GFR, glomerular filtration rate; rEPO, recombinant human erythropoietin.
Metric conversion factor: to convert meters to feet, divide by 0.3.
All analytical models were stratified by year of initiation of dialysis treatment.
Restricted to the 496,984 patients (91.8% of the overall sample) for whom complete information on weight, height, serum creatinine, hemoglobin, and albumin concentrations were available.

Winkelmayer et al., JAMA, 2009
HIF activation in CVD – importance of timing and duration

Minamashima et al., Blood 2008

Tanaka and Eckardt, Seminars in Nephrology, 2018
Key points

- HIF activation has the potential to reduce cardiovascular morbidity and mortality in CKD patients

- HIF-PHIs may have anti-inflammatory effects

- Several cardiovascular safety concerns have not been addressed yet in long-term studies

- Additional clinical studies are needed

- Acute effects may be protective – timing is critical

- Chronic HIF activation results in organ dysfunction