Pituitary Pathobiology: Clinical Relevance for Patient Management

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Henry Dukso Moon 1914-1974

- Expert in forensic and endocrine pathology
- Purified and established a biologic assay for ACTH
- Studied actions of GH and its role in carcinogenesis

Outline

- Epidemiology
- Molecular pathology
- The somatotroph example
- The FGFR4 story
- Ikaros and epigenetics
  » In development
  » In tumorigenesis
- Ikaros as an integrator of endocrine-immune-emotional status

Pituitary Adenomas: Epidemiology

- Autopsy incidence: 22.5-27%
- MRI: 20% of “normals”
- Clinical diagnosis: ?????
  » Surgical: 10% of intracranial tumors
  » Medical: ???
  » Overall: ???
  » 1.85 / 100,000 population / year
Frequency of Pituitary Tumors: Results from a Meta-analysis

- Across 7 autopsy studies - 14.4%
- Across 5 radiographic studies - 22.5%
- Overall estimated prevalence - 16.7%
- Prolactin-producing cells in \( \frac{1}{4} \) to \( \frac{1}{3} \) of tumors
- Corroborated blood hormone levels (one study)
- Most are micro (<10 mm) diameter
- Some are macro (> 10 mm) est. 1:600

Ezzat et al, Cancer 2004; 101(3):613-9

Is This An Epidemic?

- **Microadenoma:**
  Incidental finding or the cause of Cushing’s disease
- **Macroadenoma:**

Pituitary Adenomas: Why Bother?

- Variable biology
  » May be small, hormonally inactive, incidental findings
  » May be small but cause hormone excess
  » May be rapidly growing, invasive lesions
- A model of neoplasia
  » Hormone dependency

Pituitary Tumor Susceptibility Syndromes and Genes

- MEN-1 due to mutation and LOH of Menin
- McCune-Albright Syndrome due to Gs\( \alpha \) mutation
- Carney’s complex due to mutation of PRKAR1\( \alpha \)
- IFS and FIPA due to mutation of AIP
- MEN-1 like disorders due to mutations of CDKNIB/p27Kip1 or CDKN2C/p18INK4c
**Molecular Basis of Sporadic Pituitary Neoplasia**

- **Hormonal Regulatory Pathways**
  - GRH, CRH, D2R, target organ insufficiency
  - Activating mutations of Gsα in GH adenomas
- **Growth Factor Regulation**
- **Epigenetic Dysregulation**
  - Rb, CDKIs

**Transcription Factors in Pituitary Cytodifferentiation**

- Pit-1
- Estrogen Receptor
- Steroidogenic Factor-1/Lhx 4
- Thyrotrph Embryonic Factor
- Tpit, neuroD1/beta2
- Gata-2

**Pituitary Cytodifferentiation**

- Tpit
- NeuroD1/beta2

**Immunohistochemical Classification of Pituitary Adenomas**

**Major Component**
- GH-PRL-TSH
  - GH
  - SG, DG
  - GH/PRL
  - PRL
  - TSH
- ACTH
- Gonadotropin
- Unclassified

**Other Reactivity**
- Pit-1
  - α-subunit
  - Keratins
  - α-subunit, ER
  - ER
  - ?
- Tpit/neuroD1
- SF-1
- ??
**Regulatory Pathways in Pituitary Somatotrophs**

- **Stimulation**
  - GHRH
  - gsp mutations
- **Inhibition**
  - SSTR
  - ? IGF-1

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**GH-Producing Pituitary Adenomas**

- Somatotroph vs Mammosomatotroph
- Densely vs Sparsely granulated (keratin)

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**Gsp Mutations Occur in Densely Granulated Adenomas**

- GH, α-subunit + PRL
- Keratin

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**DG Adenomas Resemble Normal Somatotrophs**

- Numerous secretory granules
- Normal RER and Golgi
- Respond to SST analogues
Sparsely Granulated Adenomas

Fibrous Bodies in SGGH Adenoma

GH Receptor Alterations in SG Somatotroph Adenomas

GH Antagonist Alters Human Somatotroph Cytokeratins

Mutation of His to Leu in 5/14 and His to Arg in 1/14 SG GH adenomas

Alters receptor stability and signaling


**GH Autoregulation in Pituitary Somatotrophs**

- SG GH adenomas
- Do not respond to SST analogues
- Candidates for GHR antagonist Rx

**The Fibroblast Growth Factor Family in Pituitary Neoplasia**

- bFGF is expressed by human pituitary adenomas and the levels correlate with tumor aggressiveness
  
  *Ezzat et al, JCEM 1995;80:878-884*

- FGF4 (hst) is found in transforming DNA of human pituitary lactotroph adenomas
  
  *Gonsky et al, Mol Endocrinol 1991;5:1687-1695*

- FGFR expression is altered in human adenomas
  
  *Abbass et al, JCEM 1997;82:1160-1166*

**FGF Receptor Localization In Human Pituitary Adenomas**

- FGFR2
  - Expressed in normal
  - Lost in adenomas
  
  *Zhu et al, Am J Pathol 2007;170:1618-28*

- Epigentically silences the cancer-testis antigen MAGE-A3 (Melanoma Associated Gene Expression – Antigen 3)
  
**ptd-FGFR4: A Novel Truncated Pituitary Tumor-Derived FGFR4**

- Pituitary tumor-specific
- Constitutively phosphorylated
- Facilitates focus formation and growth in soft agar
- Facilitates tumor formation in nude mice
- Recapitulates human pituitary tumorigenesis in transgenic mice


**ptd-FGFR4 Transgenic Mice Develop Pituitary Adenomas**

- Prolactinomas since transgene is regulated by PRL promoter
- Control mice expressing wild-type FGFR4 did not develop tumors


**FGFR4 and ptd-FGFR4 Confer Distinct Invasive Properties in Vivo**

ptd-FGFR4 Alters N-cadherin Expression

- FGFR4 forms multiprotein complexes with NCAM and N-cadherin that are important for cell-cell and cell-matrix adhesion
  - Cavallaro et al, 2001
- ptd-FGFR4 interrupts these complexes, alters NCAM and N-cadherin expression and localization and reduces cell adhesion
- This explains the reticulin alterations that underlie pituitary adenomas


ptd-FGFR4 Alters NCAM/N-cadherin Complexes

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FGFR4/NCAM/ N-cadherin in Pituitary Tumorigenesis

- PD173074 – a small molecular weight FGFR tyrosine kinase inhibitor
- Selectivity based on high complementarity with the ATP-binding cavity of FGFRs
  - EMBO Journal 17:5896-5904, 1998
- Proven attenuation of FGFR4-mediated signals
  - Endocrinology 2005;146:1145-53
- Does NOT affect insulin receptor, EGF-R, PDGF-Rβ, Src, MEK, or PKC

Interrupting ptd-FGFR4
**Effect of PD173074 on N-cadherin In Primary Human Pituitary Adenoma Cells**

* Ezzat et al, Mol Endocrinol. 2006;20:2965-75

**The FGFR4 Promoter**

* Yu et al, Mol Endocrinol 2002; 16(5):1069-78

**Ikaros A Novel Pituitary Transcription Factor**

- Ikaros is one of a family of hematopoietic cell transcription factors
- Ikaros is required for T cell lineage development

* Yu et al, Mol Endocrinol 2002; 16:1069-78

**Ikaros Regulates the Endogenous POMC Gene in AtT20 Cells**

* Ezzat et al, J Clin Invest. 2005 Apr 1;115(4):1021-1029

* Ikaros is expressed in pituitary cells and tumor cell lines

* Northern blots

* ELISA (culture media)
Pituitary/Adrenocortical Function Is Impaired in Ik-/- Mice

Ezzat et al, J Clin Invest. 2005 Apr 1;115(4):1021-1029

POMC Expression is Reduced in Fetal Ik-/- Mice

Glucocorticoid Replacement Reverses Early Lethality of Ik-/- Mice

Ezzat et al, J Clin Invest. 2005 Apr 1;115(4):1021-1029

Pituitary Corticotroph Expansion is Impaired in Ik -/- Mice

Ezzat et al, J Clin Invest. 2005 Apr 1;115(4):1021-1029
**Ikaros is a Novel Pituitary Transcription Factor**

- Ik is expressed in hormone-producing pituitary corticomedullary cells
- Ik binds and regulates the POMC promoter
- Loss of Ikaros in vivo results in contraction of the pituitary corticomedullary population, reduced circulating ACTH levels, and adrenal glucocorticoid insufficiency
- The Ik/- phenotype of diminished survival is rescued by systemic glucocorticoid-hormone administration


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**Ik-/- Mice Exhibit Dwarfism**

_Ezzat et al. PNAS 2006:103(7):2214-9_

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**Growth Patterns Of Ik-Null Mice**


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**Loss of Ik leads to GH-Deficiency**

_Ezzat et al. PNAS 2006:103(7):2214-9_
**GH Restores Growth of Ik-/- Mice**

**Ezzat et al: PNAS 2006;103(7):2214-9**

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**Loss of Ikaros Impairs Pituitary Development**

**Ezzat et al: PNAS 2006;103(7):2214-9**

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**Reduced Somatotrophs in Ik-/- Mice**

**Ezzat et al: PNAS 2006;103(7):2214-9**

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**Ikaros ↓GH, ↑PRL Expression**

**Ikaros \[\downarrow\text{GH, } \uparrow\text{PRL Expression}\]**

Dr. S.L. Asa

**Distinct Effects of Ik on Pit1 Binding & Action on GH and PRL Promoters**


**Mechanisms of GH Deficiency in Ik-/- Mice**

- Direct effects on pituitary somatotrophs
  - GH gene promoter
  - other
- Indirect effects through hypothalamus or targets
  - GHRH
  - GHRHR-R

**Ik Expression in Hypothalamus**

Ezzat et al: PNAS 2006;103(7):2214-9
### Ikaros is a Novel Neuroendocrine Modulating Transcription Factor

- Ik is expressed in pituitary mammosomatotrophs and hypothalamic neurons in the arcuate nucleus
- Ik binds and regulates the GH, PRL and GHRH promoters
- Loss of Ikaros in vivo results in contraction of the pituitary somatotroph population, reduced circulating IGF-1 levels, and dwarfism
- The Ik-/− phenotype of reduced body weight is rescued by systemic GH administration

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### Ik Family

<table>
<thead>
<tr>
<th>Ik Family</th>
<th>DNA-binding</th>
<th>Dimorphism</th>
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<tbody>
<tr>
<td>E1/2</td>
<td>Repression I</td>
<td>E4, E5, E6</td>
</tr>
<tr>
<td>Ik-1</td>
<td>Repression II</td>
<td>E7</td>
</tr>
<tr>
<td>Ik-2</td>
<td>Repression II</td>
<td>E7</td>
</tr>
<tr>
<td>Ik-3</td>
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<td>Repression II</td>
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<tr>
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<td>Repression II</td>
<td>E7</td>
</tr>
<tr>
<td>Ik-8</td>
<td>Repression II</td>
<td>E7</td>
</tr>
</tbody>
</table>

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### Human Pituitary Adenomas Express Ik6

- Human pituitary adenomas express Ik6
**Immunolocalization of Ik in Human Pituitary Tissues**


**Unmasking a Cryptic Intrinsic Promoter**

ptd-FGFR4 results from transcription in intron 5 and translation start site in exon 6

Yu et al. J Biol Chem 2003; 278:19597-602

**Ik Modulates Pituitary Cell Survival**

(Ezzat et al. Molecular Endocrinology 2006; 20:2976–2986)

**Ik Mediates Pituitary Cell Apoptosis**

(Ezzat et al. Molecular Endocrinology 2006; 20:2976–2986)
Ik Isoforms Differentially Induce Bcl-XL Antiapoptotic Factor in Pituitary Cells

Ezzat et al, Molecular Endocrinology 2006, 20:2976–2986

LDL-R is Induced by Ik1


Ikaros Binds and Induces the LDL-R Promoter


Ikaros Alters Histone Acetylation & Methylation at the LDL-R Promoter

**Ikaros Regulates LDL Uptake by AtT20 Cells**


- LDL-R⁻/⁻ mice have decreased circulating levels of ACTH and contraction of the corticotroph population
- These findings expand the repertoire of Ik actions to include regulation of the cholesterol uptake metabolic pathway
- This link between tumor suppression and differentiation provides a relationship between metabolism and cancer, and has therapeutic implications for lipid-modifying drugs in Ikaros-associated cancers

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**Ikaros in Pituitary Tumorigenesis**

- Pituitary adenomas have aberrant expression of Ik-6 that acetylates the Bcl-XL promoter to up-regulate a survival signal
- Ikaros regulates LDL uptake in pituitary cells to alter tumor cell metabolism
- These data implicate Ikaros as a critical modulator of pituitary tumorigenesis

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**DNA Methyl-Transferases Mediate CDKI Changes in Pituitary Tumors**

- No evidence of mutation
- Epigenetic silencing is common
- DNMT3b expressed at higher levels in neoplastic pituitary cells
- Expression altered through histone modification
- Mediates silencing of pRb, p21, and p27 **

Zhu et al, JCEM 2008: 93:3610-3617
**Endocrine-Immune Interactions**

- **The critically ill**
  - Various hormonal dysfunctions
  - Relative adrenocortical insufficiency
  - HIV-infected patients with adrenal insufficiency
  - SARS

- **The dysfunctional immune system**
  - Recurrent infections
  - ? Increased autoimmunity
  - ? Altered sensitivity to glucocorticoids (SLE vs. RA)
  - Impaired growth

**Emotions and Endocrine Disorders**

- Acromegalic rage
- Cushing’s depression
- Prolactinoma dissociation
- Hypopituitary apathy
- Hyperthyroid mania

**Ikaros Is Also Expressed in Brain**
Mapping Ik Expression in Brain

- Ik-expressing cells are the precursors of striatal projection neurons – Medium spiny neurons (MSN)
- Ik -/- mice show no abnormalities in total number of MSN

Effects of Ik Loss in Brain

- Behavioral abnormalities
- Neurobehavioral testing was performed …
- No significant difference in:
  > the elevated plus-maze (a measure of anxiety-like behavior)
  > the acoustic startle response and pre-pulse inhibition tests (measures of motor and autonomic reaction)
  > contextual fear conditioning (measures of learning and emotion)

Catatonic-Like State in Pinch Test

- Ik-/- mice spend significantly more time in catatonia than Ik+/- littermates

**Porsolt’s Forced Swim Test: Floating Time**

A measure of depression-like behavior. Iκκ⁻⁻ mice spend significantly less time in immobility than Iκκ⁺⁺ littermates consistent with reduced behavioral despair.

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**Ikaros’ Role in Brain**

- Ikaros-mediated neuro-striatal cytodifferentiative functions impose significant and selective impact on depressive behavior.

*Kiehl et al, Experimental Neurology 2008;211:107–114*

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**Ikaros Coordinates Immune, Endocrine and Neural Development**

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**Clinical Implications & Future Predictions**

- Alterations of the Ikaros gene (mutations and/or polymorphisms) might underlie altered functions of the HPA axis and immune system, as observed together in experimental animals and humans.
- Fundamental predisposition to disorders related to stress and/or the immune response.
Thanks to ...........

• Wylie Vale
• Jeff Kudlow
• Malcolm Low
• Peter Snyder
• John Kopchick
• Daniella Rotin
• Katia Georgopoulos
• Philippe Poussier
• Ugo Cavallaro

• Lily Ramyar
• Indira Walpola
• Vince Leriche
• Ali Abbass
• Lei Zheng
• X. F. Zhu
• Jane Batt
• Megan Ward
• Rebecca DiGiovanni
• Shunjiang Yu
• Rene Mader
• Wei Liu
• Daniel Winer
• Xugong Zhu
• Sandra Fischer

• Shereen Ezzat

• Siobhan Loeper

• Tim Rasmus Kiehl