Growth Hormone Induction of Cell Growth

Michael J Waters
University of Queensland

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Somatic Cell Growth ..or

How’s the weather up there?
GH transgenic salmon: now that’s a fish!
Pig hunting in the Northern Territory

Was he on growth hormone too?
Belief: All of the growth actions of GH are mediated through IGF-1.

Are they?
GH is more effective in growth promotion than IGF-1


**TABLE 4.** Comparison of growth responses to replacement therapy with rhIGF-I of 22 children with GHRD to those of 11 children with idiopathic GHD treated with rhGH

<table>
<thead>
<tr>
<th></th>
<th>GHRD</th>
<th>GHD</th>
<th>P</th>
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</thead>
<tbody>
<tr>
<td>Growth velocity (GV)</td>
<td></td>
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</tr>
<tr>
<td>Yr 1</td>
<td>8.9 ± 1.5</td>
<td>10.9 ± 1.6</td>
<td>&lt;0.0001</td>
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<tr>
<td>Yr 2</td>
<td>6.1 ± 1.5</td>
<td>8.1 ± 2.2</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Yr 3 (6 GHRD; 8 GHD)</td>
<td>5.7 ± 1.4</td>
<td>8.3 ± 1.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Increment of GV (above baseline)</td>
<td></td>
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<tr>
<td>Yr 1</td>
<td>5.5 ± 1.4</td>
<td>8.8 ± 2.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Yr 2</td>
<td>2.9 ± 1.6</td>
<td>6.1 ± 3.1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Yr 3</td>
<td>2.9 ± 1.2</td>
<td>6.5 ± 2.4</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Change in ht SD scoreα</td>
<td>1.4 ± 0.6</td>
<td>2.2 ± 1.0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Change in ht age (HA)α</td>
<td>1.7 ± 0.8</td>
<td>2.5 ± 0.7</td>
<td>&lt;0.01</td>
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</tbody>
</table>

The difference in growth response between rhIGF-I-treated GHRD and rhGH-treated GHD groups is consistent with the hypothesis that 20% or more of GH-influenced growth is due to the direct effects of GH on bone.

rIGF-1 at 120ug/kg twice daily in GHIS, rGH at 40 ug/kg daily
IGF-1 independent contribution of GH to postnatal growth

Lupu F. et al (2001)
Dev Biol 229, 141-62
Quantification of GH, IGF-1, combined or overlap (ie GH-dependent IGF-1) and basal contributions to postnatal growth

GH does not promote growth without IGF-1 in humans

But the endogenous GH level here is high, so would we see a modest increment?

Woods KA et al (1996) NEJM 335, 1363

Serum IGF-1 undetectable
Serum GH 171 ng/ml
What does GH do to promote cell proliferation apart from IGF-1 generation?

The GH receptor is a class 1 cytokine receptor.

These cytokines stimulate cell proliferation by means of JAK and Src kinases.
Cytokine Receptor Family (Class 1)

JAK-STAT signalling
Signalling by the GH receptor

Brooks & Waters
Nat Rev Endocrinol 2010
Proliferative signalling by CA-JAK2 (V617F) in myeloid cells

ERK, PI 3K and STAT5 all contribute to JAK2 induced cell proliferation

The role of STAT5 in proliferation: is it just inducing IGF-1?

Loss of STAT5b results in loss of 50% of normal postnatal growth.

STAT5b dependent activation of the IGF-1 promoter by GH

<table>
<thead>
<tr>
<th>Stat5b</th>
<th>GH</th>
<th>Relative Luciferase Activity</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>0 1 2 3 4 5 6 7 8</td>
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<tr>
<td>---</td>
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<td>+  +  +  +  +  +  +  +</td>
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<td>+  +  +  +  +  +  +  +</td>
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STAT5b as a mitogenic factor in β-cell proliferation

Friedrichsen BN et al 2003, 2001 Mol Endo

3H-thymidine incorporation is not inhibited by IGF-1 signalling pathway blockade

DN-STAT5b inhibits 3H-thymidine incorporation

CA-STAT5b stimulates BrdU incorporation in primary β-cells
STAT5b as a mitogenic factor in β-cell proliferation
Friedrichsen BN et al 2003, 2001 Mol Endo

Induction of cyclin D2 transcript by GH
Does not require protein synthesis
(ie IGF-1 synthesis)

Cyclin D2 promoter is activated by CA-STAT5b
Induction of Myc transcript by STAT5b in BaF cells

IL-2 receptor signalling domain with only Tyr 510, responsible for STAT5 activation, and STAT5 without its terminal transactivation domain (Δ713), which does not signal.


Cycloheximide does not prevent Induction, so not IGF-1 dependent
Some other means of promoting IGF-1 independent proliferation by GH

• Induction of EGF receptor (STAT5)
• Induction of prolactin receptor (STAT5)
• Induction of LIF receptor (STAT5)
• Induction of ERα
• BMP2/4
So all GH dependent cell proliferation is not IGF-1 dependent, but *is all postnatal growth STAT5 dependent*?
GH-dependent STAT5b activation in the growth plate


Wild type rat
dw/dw rat
Activation of STAT5 by GH is decreased with fasting.
Mice with targeted knockin of mutated GH receptor:

391 mice do not activate STAT5 in response to GH

Growth of the GHR Knock-in mice

Male mice

- WT
- 569
- 391
- GHRKO (42%)

Female mice

- WT
- 569
- 391
- GHRKO (51%)
JAK2-contributes the STAT5 remainder to postnatal growth via ?PI 3K

Hepatic IGF-1 transcript

Serum IGF-1

<table>
<thead>
<tr>
<th>Wt</th>
<th>Box 1 Delete</th>
</tr>
</thead>
<tbody>
<tr>
<td>569</td>
<td>391/-/-</td>
</tr>
</tbody>
</table>
ERK and Src activation by GH is normal in Box 1 delete mice, so ERK alone does not contribute to GH-dependent growth.

IB: pMAPK
IB: p44 MAPK

WT
Box1Δ Mutant
GH
+  -  +  +  +  -  -  -

IB: pMAPK
IB: p44 MAPK

WT
Box1Δ Mutant
GH
-  +  -  -  +  +

IB: P-Src
IP: Src
IB: Src

Linda Kerr

Even Dogs Do IT...

Tell her you are a guard dog. Yeah, a Rottweiler, and that you're single and neutered....
Is there more to the story of GH and cell proliferation?

Becky Conway-Campbell
Immunoreactive GH Receptors are present in the nucleus from the first cleavage in mouse embryos

Pantaleon M et al (1997) PNAS
Nuclear Localisation of GHR in regenerating liver after partial hepatectomy (pHx)

Conway-Campbell BL et al (2007) PNAS

Control rat liver

24 hr pHx

GHR Texas red
PCNA FITC
DAPI
Merge
Merge+
Dapi

From 600 nuclei each

$\rho = 0.7749$

HepatX 53% mitotic

$\rho = 0.6846$

Control 2.5% mitotic
Nuclear GHR is evident in proliferating BaF cells but absent in quiescent cells.

Growth media + GH

Starve media - GH

Conway-Campbell BL et al (2007) PNAS
GHR undergoes GH-regulated nuclear transport in BaF-GHR

Confocal Laser Scanning Microscopy
WT-GHR Baf 10 hour serum starve

Before and after treatment with 5 nM hGH for 10 min
The NLS of SV40 T-ag increases nuclear targeting of the GHR in BaF/3 lines in the absence of GH.
Effects of increased GHR nuclear targeting on proliferation
$^{3}$H-Thymidine incorporation assay for proliferation of BaF–NLS-GHR clones in serum – largely independent of exogenous GH.
Constitutive activation of STAT5b is evident in rb-NLSGHR-BaF, not h-NLSGHR-BaF

This is blocked by inhibition of JAK2
Inhibition of JAK2 blocks constitutive proliferation in rb-NLSGHR-BaF.

Human NLS-GHR does not display constitutive proliferation.

Could the cells be sensitized to endogenous (mouse) GH?
Nuclear localizing the receptor sensitizes the cell to GH

Total receptor number matched by Scatchard
BaF cells synthesise GH, and cells with nuclear GHR respond to this because of their increased sensitivity.
Proliferation and survival genes activated by nuclear localisation of GHR

<table>
<thead>
<tr>
<th></th>
<th>NLS</th>
<th>WT</th>
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<tbody>
<tr>
<td></td>
<td>0</td>
<td>1</td>
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</table>

Cish

Mybbp1a

Svn

18S

Graphs showing the fold change from WT 0 for Cish, Survivin, and MybBP 1a under GH treatment (hr) for NLS and WT conditions.
Unable to move from the spot, Trevor realises that he’s buried the wrong nuts.
Identification of nuclear proteins which bind to the GH receptor
Nuclear proteins which associate with the ECD: Affinity chromatography with hGHR ECD
<table>
<thead>
<tr>
<th>Unknown</th>
<th>ID</th>
<th>accession number</th>
<th>mammalian homolog</th>
<th>Function</th>
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<tbody>
<tr>
<td>P31</td>
<td>RPL4</td>
<td>AAA20990.1</td>
<td>L7A / TRUP</td>
<td>Ribosomal protein</td>
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<tr>
<td></td>
<td></td>
<td>GI:172444</td>
<td></td>
<td>Transcriptional regulation</td>
</tr>
<tr>
<td>P33</td>
<td>Sbp1</td>
<td>NP_011829.1</td>
<td>RNA binding motif protein 14; SYT interacting protein (SIP); coactivator activator (CoAA)</td>
<td>RNA binding</td>
</tr>
<tr>
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<td></td>
<td>GI:6321753</td>
<td></td>
<td>Splicing factor</td>
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<td></td>
<td></td>
<td></td>
<td>Transcriptional regulation</td>
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<tr>
<td>P50</td>
<td>EF1 alpha</td>
<td>NP_015405.1</td>
<td>EF1 alpha</td>
<td>Translational regulation</td>
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<tr>
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<td>GI:6325337</td>
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<tr>
<td>P160</td>
<td>nucleoporin</td>
<td>CAA83584.1</td>
<td>Nup145</td>
<td>Nuclear import</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GI:496731</td>
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</table>

**GH Receptor ECD Interactors:**
Results of MS/MS

**KNOWN** interaction observed in | Function
--- | ---
Ran / ARA24 GST pulldown | nuclear import transcriptional regulation
Importin α in vitro Eliza | nuclear import
Importin β in vitro Eliza | nuclear import

**GH Receptor ECD Interactors:**
GST pulldown and candidate approach

- CoAA potently coactivates transcription through its interactions with both the TRBP activation domains and RNA-containing transcriptional complexes.

- CoAA acts as an RNA splicing factor

- CoAA has Nuclear Localization Sequences
CoAA associates with GHR in a GH-dependent manner
Stable transfection with CoAA increases the maximum mitogenic response to GH

Populations FACS sorted for GHR expression +/- CoAA transfection
1. Association of bound GHR to CoAA with Imp-α/β complex

2. Docking at NPC

3. Translocation through NPC by the complex

4. Dissociation of whole complex by GTP hydrolysis to GDP

Proliferative Signalling by GH
Role of the growth hormone–IGF-1 axis in cancer

Yash Chhabra*, Michael J Waters†‡ and Andrew J Brooks†

*These authors contributed equally to this article

A substantial body of evidence supports a role for the growth hormone (GH)–IGF-1 axis in cancer incidence and progression. This includes epidemiological evidence relating elevated plasma IGF-1 to cancer incidence as well as a lack of cancers in GH/IGF-1 deficiency. Rodent models lacking GH or its receptor are strikingly resistant to the induction of a wide range of cancers, and treatment with the GH antagonist pegvisomant slows tumor progression. While GH receptor expression is elevated in many cancers, autocrine GH is present in several types, and overexpression of autocrine GH can induce cell transformation. While the mechanism of autocrine action is not clear, it does involve both STAT5 and STAT3 activation, and probably nuclear translocation of the GH receptor. Development of a more potent GH receptor antagonist or secretion inhibitor is warranted for cancer therapy.

Keywords: autocrine GH • breast cancer • colon cancer • epidemiology • GH receptor • growth hormone • insulin-like growth factor-1 • nuclear GH receptor • prolactin • prostate cancer • signal transducer and activator of transcription-5

Six decades of research have established that growth hormone (GH) plays a crucial role in promoting proportionate postnatal growth, and that this is largely mediated through the induction of IGF-1. This action is supported by important roles of GH in the regulation of metabolism. The GH–IGF-1 axis promotes neoplasia. As discussed here, converging data from recent epidemiologic, animal and in vitro studies indicate that the state of the GH–IGF-1 axis has important influences on cancer biology, cancer risk and carcinogenesis.
Thanks, its time for Coffee!

m.waters@uq.edu.au