Pediatric RPAC Educational Day (Feb. 16, 2000)

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- Growth Hormone Action: From Mouse to Man
Growth Hormone
Structure/function studies

- “Change the structure and assay the alteration in function”
Growth Hormone Biological Activities

- Growth
- Lactation
- Metabolism
  - Bone
  - Fat
  - Muscle
  - Liver
  - Kidney
  - Others
Dose dependent decrease in fat, and increase in muscle and bone!!!
Amino acid sequence of bGH with four α-helices indicated

Signal peptide cleavage sites
GH Genes

One can insert GH genes into cultured mouse cells or fertilized mouse eggs
Mouse L cell expressing GH
Pre-microinjection

Post-microinjection

Male pronucleus
GH transgenic mice
GH Crystal Structure

2.5 Angstrom Resolution

Blue + N terminus
Red = C Terminus
Light green = helix 3
Experimental Protocol

- In vitro mutagenesis of GH gene or cDNA
- Oligonucleotide sequencing of mutations
- Expression of mutated DNA in mammalian cell culture
- Purification of GH analog
- Receptor Binding studies
- GH Responsive cell lines
  - Preadipocytes
  - Engineered GHR cell lines
  - NB₂
  - IM9
- Production of transgenic animals
  - Growth parameters
  - Morphometrics
  - Endocrine and physiological studies
  - Histological Studies
- Animal models
Growth Hormone

- pGH - porcine
- bGH - bovine
- oGH - ovine
- hoGH - horse
- rGH - rat
- aGH - avian
- hGH - human
- mGH - monkey

Amino acids and their positions in the bGH 109-126 sequence:
Hydrophobic Amino Acids

bGH 109-126

Glu 111
Glu 118
Arg 125
Lys 114
Gly 119
Gly 118
Ala 122
Asp 115
Glu 126
Glu 118
Glu 111
Glu 117
Tyr 110
Leu 121
Ile 120
Val 109
Leu 113
Leu 116
Leu 113
Leu 123
Leu 124
Met 124

Hydrophobic
Hydrophilic Amino Acids

bGH 109-126
Imperfect amphipathic α-helix
Targeted changes

![Diagram showing targeted changes in proteins with specific amino acid positions and values.](image-url)
bGH 109-126
“Perfect” amphipathic $\alpha$-helix
GH Transgenic Mice
4 fold larger than control
GH M8 Transgenic Mouse
2 fold smaller than control
bGH 109-126
bGH
109-126
One amino acid change, i.e. glycine to arginine results in a growth inhibitor or "growth hormone antagonist"
Not all is well with these animals

GH transgenic animals die prematurely
GH Transgenic Mice

Control Mice

COLLIV Antiserum

Glomerulosclerosis - scarring of the glomerulus
Gly 119 and Ala 122 – one helical turn apart!!!!!!!
Any amino acid at position 120, other than alanine, results in a GH antagonist.
Second target model of GH action - 1990
Site 2

deVos, et al 1992
GHR dimer

Gly 120 = yellow
Trypt 104 = white
Clinical uses of a Growth Hormone Antagonists

- Acromegaly
- Diabetes
- Cancer
Acromegaly

- Increased GH levels due to pituitary gland tumor
- High circulating levels of IGF-1
Potential Problems with a growth hormone antagonist in the clinic

- Short half-life
- Antibody formation
Rational design of B2036-PEG

Site 1
- 8 mutations
- Increases binding to 1st GHR

Site 2
- 1 mutation
- Prevents binding to 2nd GHR

Pegylation
- Increases half-life
Individual IGF-I levels before and after B 2036-PEG
GH Antagonists and Diabetes Induced End Organ Damage

Kidneys and Eyes
Dwarf Mice are Protected from Glomerular Hypertrophy

![Graph showing glomerular volume comparison between SJL and Dwarf mice under DM(-) and DM(+) conditions.](image-url)
Dwarf Mice Are Protected From Diabetic Glomerulosclerosis

<table>
<thead>
<tr>
<th>Sclerosis Index</th>
<th>SJL</th>
<th>Dwarf</th>
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<tbody>
<tr>
<td>DM (-)</td>
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<tr>
<td>DM (+)</td>
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The graph shows that compared to SJL mice, Dwarf mice have a lower sclerosis index under both diabetic (DM) conditions (- and +).
Transgenic Dwarf Mice Do Not Develop Diabetic Nephropathy

Control

Control + STZ- DM

Dwarf (G119K)

Dwarf + STZ- DM
Make “control” animals diabetic, then treat with the GH antagonist.

GH antagonists mice are protected from diabetes induced glomerulosclerosis.
Retinopathy - excess hGH action

- Neovascularization, microaneurysms, fluid leakage, blindness
- Frequently associated with diabetes and prematurity (most common cause of blindness in U.S. and Europe)
- Laser photocoagulation only therapy
- Linkage to excess hGH?
GH and Retinal Neovascularization

- GH, GH antagonists, and controls
- Expose 7 day old mice to 75% oxygen
  - induces retinal vaso-obliteration
  - return to room air at day 12
  - check for retinal neovascularization through day 17
  - Extensive neovascularization occurs in 100% of wild type mice
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Support

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USDA
"Structure-Function relationships of growth hormone and other members of the growth hormone family", Handbook of Physiology (Hormonal Control of Growth), Chapter 6, Kopchick and Chen, 137:145-162, 1998.