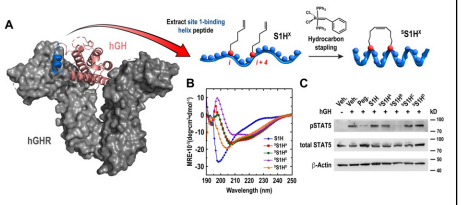
https://doi.org/10.17952/38EPS.2026.P487

A Helical Peptide Antagonist of the Human Growth Hormone Receptor

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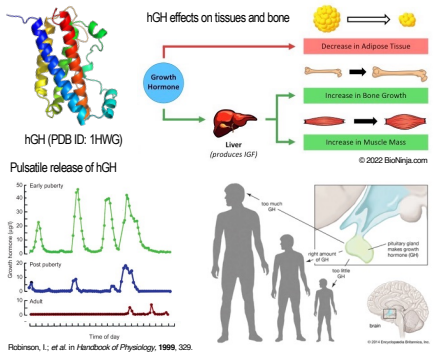
Abstract



The binding of human growth hormone (hGH) to the human growth hormone receptor (hGHR) is a key endocrinological process that controls critical aspects of cell growth, proliferation and differentiation. This sequential, asymmetric binding event involves the interaction between a single hGH molecule and distinct sites (site 1 and site 2) on the extracellular domain of a preformed hGHR homodimer. Our group recently identified S1H, a rationally-designed peptide sequence mimetic of the hGH site 1-binding helix that disrupts the hGH-hGHR interaction and inhibits hGH-mediated STAT5 phosphorylation in cultured hGHR-positive cell lines. Structure-activity relationship studies revealed a strong correlation between helical propensity and inhibitory potency of the S1H peptide, prompting the design of structurally "stabilized" S1H variants (SS1H) with improved helicity (Figure A). Here, we used hydrocarbon stapling to generate a series of ^{S}S1H peptides that proved to be more helical, proteolytically stable and biologically active compared to linear (unstructured) S1H (Figures B and C). Notably, one ^{S}S1H derivative, ^{S}S1H^{B}, inhibited hGH-induced STAT5 phosphorylation in hGHR-positive bladder cancer cells more effectively than pegvisomant, the only hGHR antagonist currently approved by the FDA. Collectively, our results demonstrated that hydrocarbon stapling improves the antagonistic effects of S1H peptides and elevates their potential as chemical probes to study the molecular mechanisms of hGH signaling. It is also anticipated that ^{S}S1H peptides will serve as potent lead compounds for developing next-generation therapeutics designed to treat endocrine disorders that manifest along the hGH-hGHR signaling axis.

1

Biology of the Human Growth Hormone (hGH)



Robinson, I.; *et al. in Handbook of Physiology*, **1999**, 329.

Growth Hormone Actions in Humans

Normal Physiology

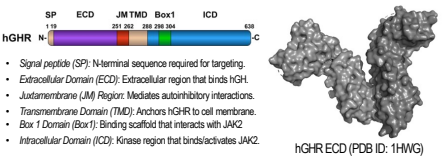
- **Promotes bone and muscle growth**
- **Supports insulin resistance**
- **Stimulates lipolysis in adipose tissue**

Disease and Medicine

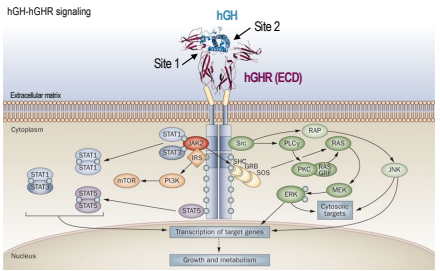
- **Excess growth hormone can lead to gigantism or acromegaly**
- **Growth hormone deficiency (GHD)**
- **Used to increase muscle mass, reduce body fat, and aid recovery**

2

The Human Growth Hormone Receptor (hGHR)



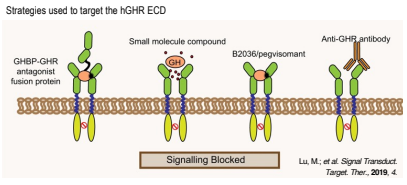
- *Signal peptide (SP)*: N-terminal sequence required for targeting.
- *Extracellular Domain (ECD)*: Extracellular region that binds hGH.
- *Juxtamembrane (JM) Region*: Mediates autoinhibitory interactions.
- *Transmembrane Domain (TMD)*: Anchors hGHR to cell membrane.
- *Box 1 Domain (Box1)*: Binding scaffold that interacts with JAK2
- *Intracellular Domain (ICD)*: Kinase region that binds/activates JAK2.

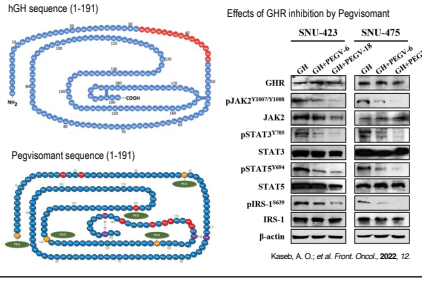


Brooks, A.; *et al. Nat. Rev. Endocrinol.* **2010**, *6*, 515.

3

Inhibiting the hGH-hGHR Signaling Axis

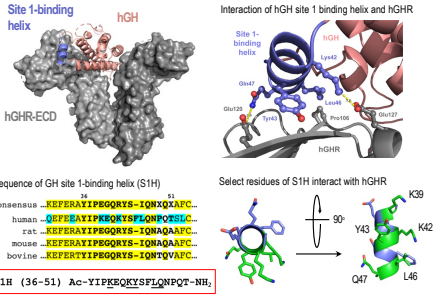




Kaseb, A. O.; *et al. Front. Oncol.*, **2022**, *12*.

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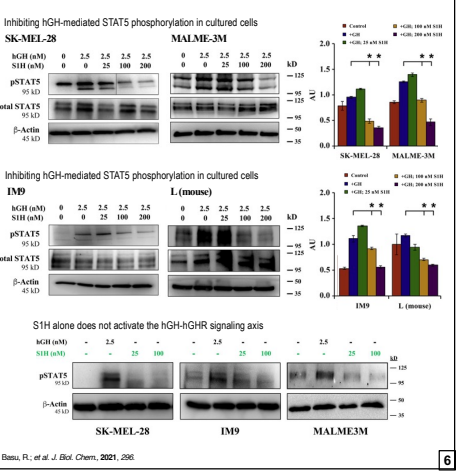
Design and Characterization of S1H Peptide



Basu, R.; *et al. J. Biol. Chem.*, **2021**, *296*.

5

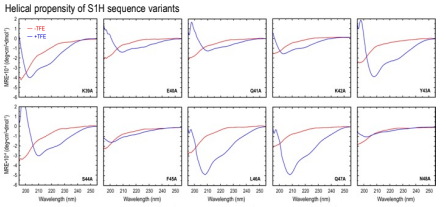
S1H Inhibits hGH-mediated STAT5 Phosphorylation

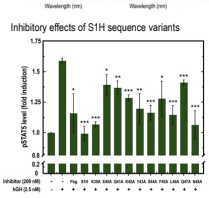


Basu, R.; *et al. J. Biol. Chem.*, **2021**, *296*.

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Correlating Helicity and Inhibitory Activity





Structural and inhibitory data of S1H sequence variants

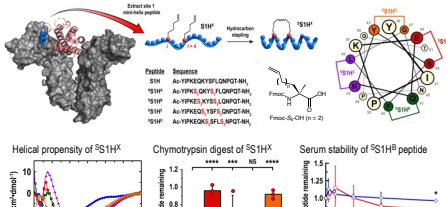
Peptide	Sequence	% helical peptide - TFE	% helical peptide + TFE	Percent inhibition
S1H (36-51)	^{36}YIPKEQKYSFLQNPQT-NH2	1.1	48.2	93.3
S1H (K36A)	^{36}YIPAEQKYSFLQNPQT-NH2	23.4	67.1	88.5
S1H (E40A)	^{36}YIPKAQKYSFLQNPQT-NH2	5.7	28.6	93.4
S1H (Q41A)	^{36}YIPKEAKYSFLQNPQT-NH2	8.3	25.4	97.4
S1H (K42A)	^{36}YIPKEQAYSFLQNPQT-NH2	2.4	24.4	91.7
S1H (Y43A)	^{36}YIPKEQKASFLQNPQT-NH2	11.2	68.8	66.6
S1H (S44A)	^{36}YIPKEQKYAFLQNPQT-NH2	15.7	63.5	72.3
S1H (F45A)	^{36}YIPKEQKYSALQNPQT-NH2	11.1	21.4	92.5
S1H (L46A)	^{36}YIPKEQKYSFAQNPQT-NH2	7.0	66.3	79.1
S1H (Q47A)	^{36}YIPKEQKYSFLANPQT-NH2	8.2	66.2	90.1
S1H (N48A)	^{36}YIPKEQKYSFLQAPQT-NH2	6.7	12.2	89.1

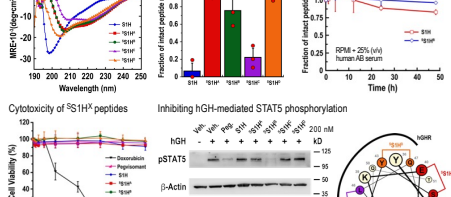
- Helical propensity for each variant was determined in the presence of TFE, a structure-inducing co-solvent.
- Inhibitory activity was evaluated by co-treating SK-MEL-28 cells with peptides and hGH and quantifying pSTAT5.
- Results showed a strong correlation between helical propensity and inhibitory function, *structured S1H variants?*

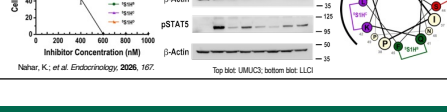
Basu, R.; *et al. J. Biol. Chem.*, **2021**, *296*.

7

Characterization of ^{S}S1H^{X} Peptides







Nahar, K.; *et al. Endocrinology*, **2026**, *167*.

Top blot: UMUC3; bottom blot: LLC1

8

Conclusions and Future Directions

What have we learned?

- We developed a novel peptide-based antagonist (S1H) of the human growth hormone receptor.
- S1H was derived from the hGH site 1-binding helix; inhibits hGH-mediated STAT5 phosphorylation.
- SAR studies revealed a strong correlation between helical propensity and inhibitory function.
- Select stapled S1H derivatives show enhanced helicity, proteolytic stability, and biological activity.

…and where can we go from here?

- Purify recombinant hGHR from bacteria for *in vitro* binding assays; map S1H-hGHR interaction.
- Testing the activity of ^{S}S1H^{B} *in vivo* (*e.g.* lowering hGH-mediated IGF-1 levels in mice).
- Develop scyllatoxin-based S1H derivatives; more stable structure and genetically encodable.
- Develop S1H derivatives as *agonists* of the hGHR; linked peptides for hGHR dimerization.

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38th EPS European Peptide Symposium
6 - 11th of September 2026
Vienna, Austria

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