Kisspeptin treatment in mesenchymal bone cells induces markers of inflammation

ABSTRACT

Kisspeptin together with its receptor, GPR54 (Kiss1R), are known for their function as the principal regulators of the onset of mammalian puberty. Given the characteristic growth spurt and bone development that occurs during puberty, recent studies have provided insights into the role kisspeptin has in the control of skeletal homeostasis as well as bone cell differentiation. Both kisspeptin and Kiss1R knockout mice display decreased trabecular bone mass. The present study hypothesizes kisspeptin communicates with bone cells which may aid to regulate growth and differentiation. The objective of this work was to establish the expression patterns of kisspeptin and Kiss1R in bone and uncover for the cell signaling networks it can affect. Mesenchymal bone cells, W-20-17 (W20), were analyzed via western blot analysis and found to express Kiss1R. W20s were then treated with the kisspeptin peptide (50μM) for approximately 18 hours in starving media. Signal transduction antibody microarrays were run to compare changes in the intracellular signaling networks affected by kisspeptin treatment (n=3). Of the 165 specific antibodies targets related to signaling cascades, 23 targets were significantly increased or decreased ($p<0.05$). Interleukin-5 (IL-5) and Tumor necrosis factor-α (TNF-α) expression was significantly increased by kisspeptin treatment (1.86 and 1.24 respective fold; $p<0.05$). Work is ongoing to identify if this response is Kiss1R-dependent. These data suggest kisspeptin treatment can induce an inflammatory response in bone.

MATERIALS & METHODS

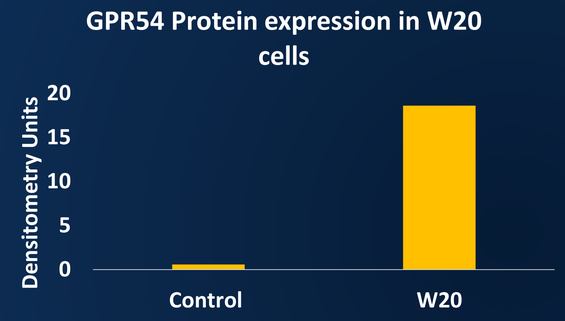
W-20-17 (W20), analyzed via western blot analysis for GPR54

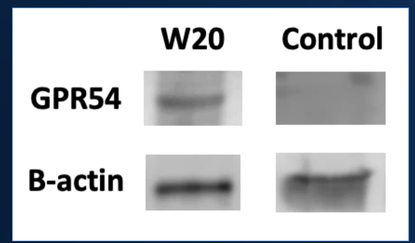
- *Cell Culture* - W20 cells were cultured for normal maintenance in DMEM, combined with 10% (v/v) FBS, 1% (v/v) Antibiotic-Antimyotic Solution (100x), and 1:5000 (v/v) puromycin.
- *SDS-PAGE:* Standard PageRuler Plus Prestained Protein Ladder and W20 samples were loaded on polyacrylamide 12% Mini-PROTEAN gel at 30 ug, protein concentrations determined by BCA assay.
- Rabbit Polyclonal Anti-Kisspeptins Receptor antibody (OriGene) were used to probe membranes at a dilution factor of 1:1000. Mouse polyclonal anti-b-actin 1% antibodies were then used to probe as a loading control at a dilution factor of 1:2000. Chemiluminescent Detection Reagent from the Advansta Western was used for blotting detection. Blot image density was analyzed using ImageJ via NIH.

W-20-17 (W20) cells were treated with kisspeptin and analyzed by signal transduction array

- W20 cells were treated with either 50 μM kisspeptin in starving media and incubated for approximately 18 hours or PBS control. Cells were then harvested, pelleted, and then lysed for protein isolation. BCA assay was used to determine total protein concentration. Samples from both the treatment and control group (50 μg) were run on the Full Moon BioSystems Signal Transduction Antibody Array. Results were normalized to b-actin. Data are presented as mean fold change +/- standard error of the mean (SEM) of each signal transduction protein target relative to b-actin from n=3 sample replicates.

A mesenchymal bone cell line expresses kisspeptin receptor protein





	Average Fold Change	Standard Error of the Mean	P-value
Amylin Peptide	1.73	0.13	0.01
Granzyme B	1.81	0.02	0.000001
HIF-1a	2.22	0.16	0.002
IL-5	2.38	0.21	0.003
IL-6	1.82	0.12	0.002
NOS-i	1.63	0.05	0.0002

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RESULTS

The mesenchymal bone cell line (W20) expresses kisspeptin receptor protein

- Kiss1R (GPR54) protein expression was detected in W-20-17 (W20). Densitometry results from western blots were generated from ImageJ.

Kisspeptin-treated mesenchymal bone cells increase the expression of markers of inflammation

- Antibody arrays were run on protein samples collected from both kisspeptin-treated and control mesenchymal bone cells (W20) in three separate runs (n=3). Samples were normalized to beta-actin.
- Of the 165 specific antibody targets related to signal transduction, 6 targets had a statistically significant change in protein expression ($p<0.01$).
- To determine if protein expression results were statistically significant, average fold change was normalized via T-test to beta-actin, p-value was set to <0.01, and protein expression was said to be increased if average fold change was >1.50 or decreased if average fold change was <0.50. Of the 165 proteins tested, 6 pertinent proteins were selected to be highlighted.
- Of interest, interleukin-5 (IL-5), interleukin-6 (IL-6), and granzyme B expression were significantly increased by kisspeptin treatment (2.38, 1.82, and 1.81 respective fold change; $p<0.01$). Also of interest, Hypoxia-inducible factor 1-alpha (HIF-1a, HIF-1a), amylin peptide, and nitric oxide synthase type 1 (NOS-i) were significantly increased with kisspeptin treatment (2.22, 1.73, and 1.63 respective fold change; $p<0.01$).

FUTURE DIRECTIONS

- Future work will quantify expression of Kiss1R by qPCR in W20 cells and other bone cell lines.
- Work to identify if the findings of the signaling array are Kiss1R-dependent are underway and the elucidation of the mechanism(s) by which these findings occurred.
- This work is cause for speculation that kisspeptin may play a role in the inflammatory response of bone.