Leptin Influences FGF2 in Culture-Dispersed Adipose Tissue for Angiogenic Progression

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Abstract

The process of wound healing requires the transportation of substrates needed for the rebuilding of tissue through appropriate vessel formation; angiogenesis. Adipose-tissue-extracts, applied to wounds has been reported to accelerate the wound healing process. These properties are likely attributed to one or more factors such as stem cells, dense vascularization, and are regulated through growth factors such as vascular endothelial growth factor (VEGF), angiopoietin, fibroblast growth factor (FGF2), and leptin. Both leptin and FGF2 are utilized in the biomedical community to facilitate the wound healing process. Predominantly synthesized and secreted by mature adipocytes (MA), FGF2 and leptin are just two of the many potent growth factors utilized in the process of angiogenesis. Leptin has been found to influence vascular endothelial cell (VEC) elongation and subsequent tube formation in adipose tissue. FGF2 also facilitates VEC morphological progression. Therefore, it was hypothesized that leptin and FGF2 have an established relationship in regards to angiogenic progression. Adipose tissue was collected from 4 prepubertal female pigs 148 ± 12 days of age weighing 86 ± 6kg. MA in treatment groups with or without leptin (10^{-11}) or rabbit polyclonal anti-leptin (1:100 dilution) underwent cell culture to identify morphogenic progression stages: elongation, sprouting, nascent tube, and full vessel tube formation. mRNA was then isolated from each cultured treatment group and RT-PCR was conducted to assess the expression of target genes of leptin receptor (Ob-Rb), and FGF2 receptor (rFGF2). The MIXED procedure of SAS was utilized to determine the effect of treatment on gene expression of target genes in-vitro. Leptin tended (P=0.1) to increase FGF2 (128.0±15.5% vs 100.0±0.0%, -11M vs. 0M) expression in pool-cultured cells and numerically increased ObRb in leptin. Blocking leptin with antibody did not mitigate the effect of leptin. Leptin does influence FGF2 in cultured-dispersed adipose tissue. Isolating leptin treated cells exhibiting morphologic changes would reveal the magnitude of FGF2 expression.

Introduction

Wound healing is an intricate process that is initiated after a trauma transpires and is executes in 3 stages: inflammation, proliferation, and remodeling (1,2). The inflammatory stage includes coagulation of blood and an influx of leukocytes to the trauma site to protect against infection. The proliferative stage is responsible for wound closure, which includes fibroplasia, re-epithelialization, and angiogenesis. In the remodeling stage, the trauma site is restructured to resemble the original tissue organization. Critical to all 3 stages is appropriate re-vascularization (angiogenesis), which transports nutrients, cytokines, leukocytes, and waste to and from the wound site. Adipose tissue extracts applied to wounds has been reported to accelerate the wound healing process (3). Adipose tissue wound healing properties are likely attributed to one or more factors such as multiple growth factors, stem cells, and dense vascularization. Leptin is a predominant hormone and is synthesized and secreted by MA's. Leptins numerous properties include being a main adipokine, possessing inflammatory factors, and inducing the synthesis and secretion of proteases and adhesion molecules needed for the progression of angiogenesis (4). FGF2, also a predominant hormone synthesized and secreted by MA's, also facilitates VEC morphological progression. Mature adipose tissue and extracts have been utilized to augment rate of healing in wound tissue with attribution to FGFs.

Materials & Methods

- **Animals**
  - Four crossbred (Yorkshire x Hereford x Hampshire) pre-pubertal female piglets, ~150d of age.
- **Tissue Collection**
  - Piglets were anesthetized and subcutaneous adipose tissue (3 grams) was harvested from the cervical vertebral region.
  - Tissue Transport: Harvested adipose tissue were placed in pre-warmed (37°C) Hanks solution (HyClone; Logan, UT) for transportation. Adipose tissue was enzymatically digested for cell culture.

Materials & Methods

- **Cell Preparation**
  - **Cell Isolation**: Tissue was digested in phenol-free Dulbecco's Modified Eagle's Medium Nutrient Mixture F12 Ham (DMEM; Sigma Aldrich; St. Louis, MO) with Type II Collagenase (1.5 mg/ml; Sigma) in a vigorously shaking water bath at 37°C for 60 min.
  - **Cell separation**: vascular endothelial cells (VEC), fibroblasts and preadipocytes (all other cells; AOC) were separated from mature adipocytes (MA, floating cells) for pre-treatment culture.
- **Cell Culture:**
  - **Pre-treatment Cultures:**
    - AOC Attachment cultures:
      - AOC (~8.0 x 10^5 cells/well) were cultured in triplicate for 24 hr in attachment media [phenol-free DMEM, 10% charcoal stripped fetal bovine serum (CFBS, HyClone), 0.1 mg/ml streptomycin, 100 U/ml penicillin, 2.5 mM L-glutamine (HyClone)] at 37 ºC in an atmosphere of 5% CO_2 in air with 95% humidity to promote cellular aggregation and attachment.
      - Following 24 hrs, cultures were analyzed for evidence of angiogenic progression.
    - MA Acclimation culture:
      - MA (~3.0 x 10^5 cells/well) were cultured in triplicate in standard non-attachment media [phenol-free DMEM, 0.1 mg/ml streptomycin, 100 U/ml penicillin, 2% CFBS, 1.5% bovine serum albumin, 2.5 mM L-glutamine] at 37 ºC in an atmosphere of 5% CO_2 in air with 95% humidity for 24 hr to acclimate cells to culture conditions prior to treatment.
  - **Treatment Cultures:**
    - Attachment media was removed from AOC cultures and replaced with standard media. MA cells were added to AOC cultures w/without treatment. Treatments were 1) 0M (control, media only), 2) porcine Leptin (10^{-11}, Cell Sciences), or 3) rabbit polyclonal Leptin Antibody (Leptin Ab, Thermo Fisher). Antibody treatment was utilized to inhibit endogenous Leptin to confirm that MA influenced angiogenesis through Leptin. Cells were incubated for 24 hr under the same incubation conditions. At termination, angiogenic progression was documented.
- **Angiogenic Progression Analysis**
  - **Evidence of VEC angiogenic progression:**
    - VEC were categorized relative to the state of progression: *Elongation* (elongated and tapered on 2 ends), *Sprouting* (cytoplasmic pseudopod projections, 3-or more), *Nascent Tube* (sprouts from one VEC connecting to a sprout from another VEC), *Tube formation* (cylinder structure resembling the beginning formation of a lumen).

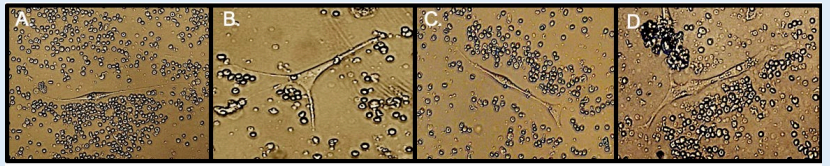

Figure 1. Representative pictures of cells during angiogenic progression. A: Elongation. B: Sprouting. C: Nascent Tube. D: Tube formation

- **Statistical Analysis:**
  - The MIXED procedure of SAS was utilized to determine the effect of MA addition on angiogenic progression and the effect of Leptin and Leptin Ab on angiogenic progression. The PDIFF option was conducted to identify significant differences among treatment means.
  - All means are reported as LS MEANS±SEM.

Materials & Methods

- **RNA Isolation**
  - **RNA Extraction:** Tissue underwent two-day RNA extraction to isolate mRNA from treatment group samples..
- **Laboratory Diagnostic Testing**
  - **RT-PCR Analysis:** used to determined target gene expression of Ob-Rb (leptin receptor) and FGF2 (FGF2 receptor)
- **Statistical Analysis:**
  - The MIXED procedure of SAS was utilized to determine the effect of leptin on FGF2 expression in dispersed adipose tissue.. The PDIFF option was conducted to identify significant differences among treatment means.
  - All means are reported as LS MEANS±SEM.

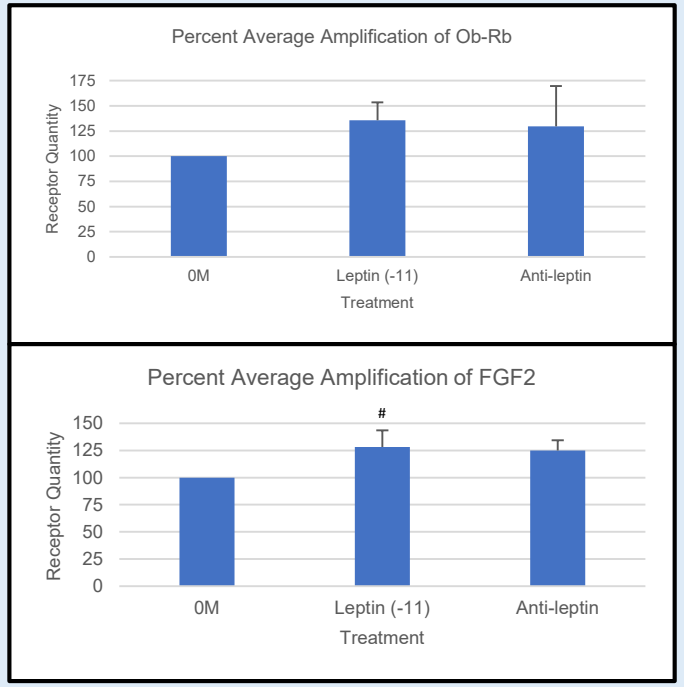

Figure 2. Percent Average Amplification of Ob-Rb in Culture-Dispersed Adipose Tissue. Percent Average Amplification of FGF2 in Culture-Dispersed Adipose Tissue. #: Mean compared to 0M tends (P=0.1) to differ.

Conclusion

- Leptin tended to increase FGF2 expression in pool-cultured cells and numerically increased Ob-Rb in leptin
  - Blocking leptin with antibody did not mitigate the effect of leptin.
  - Leptin does influence FGF2 in cultured-dispersed adipose tissue
  - Isolating leptin treated cells exhibiting morphologic changes would reveal the magnitude of FGF2 expression

Acknowledgements

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References

1. Wang PH, Huang BS, Horng HC, and Yeh CC. Wound healing. J Chin Med Assoc. 2017; 81(2018): 94-101.
2. Oliveira-Gonzalez, AC, Fortuna, T, Aruajo, Z, and Ribeiro, A. (2016). Wound healing – A literature review. An Bras Dermatol. 91(5):614-20.
3. Piccolo NS, Piccolo MS, Piccolo MTS. Fat grafting for treatment of burns, burn scars, and other difficult wounds. *Clin Plast Surg.* 2015;42(2): 263-283.
4. Gonzalez-Perez RR, Viola L, and Newman G. Leptin's pro-angiogenic signature in breast cancer. 2013; 5:1140-1162.