Observing Mature Adipocytes When Treated with FGF2 Antibody

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Abstract

When an injury occurs, there is a process of healing referred to as angiogenesis. Angiogenesis is comprised of a matrix of complex rebuilding from preexisting cells and contributes to the formation of vascular endothelial cells (VEC). These cells are a crucial role in the process of angiogenesis. The main storage of adipocyte cells can be found in fat tissue which consists of mature adipocytes that aid in the regeneration of cells. In this quantitative study fat tissue from three Brangus male cattle was collected and the mature adipocytes (MA) were then isolated from the stromal vascular fraction (SVF)of the tissue. SVF were cultured for 24 hr to promote plate adhesion. MA were added after 24hr with fibroblast growth factor-2 (FGF2) polyclonal antibody (1:50 or 1:100 dilution) or without antibody and cultured for an additional 24 hr. Blocking endogenous FGF2 reduced (P=0.01) early morphological progression of VEC in the angiogenic process.

PURPOSE

This study's purpose is to address the question if FGF2 can influence the morphology change of VEC and/or the growth of the cells while providing further insight into the cell development in bovines.

METHODS

➢**Animals**

- Three Brangus steers of similar age and weight were utilized for this study (IACUC approval #2023-06-23).

➢**Tissue Collection**

- An epidural was conducted by administering a local sedative (Lidocaine 2%HCL) between the 3rd and 4th caudal vertebrae to anesthetize the tail head region. A shallow incision was made to collect ~3 g of subcutaneous adipose tissue.
- Tissue Transport: Harvested adipose tissue was placed in a pre-warmed (37° C) HANKS solution for transportation. Adipose tissue was enzymatically digested for cell culture.

➢**Cell Preparation**

❑Cell Isolation: Tissue was digested in phenol-free Dulbecco's Modified Eagle's Medium Nutrient Mixture (DMEM) with Type II Collagenase (1.5 mg/ml; Sigma) in a shaking water bath at 37° C for 90 min.

❑Cell separation: Stromal vascular fraction (SVF: VEC, fibroblasts, pericytes, smooth muscle cells, preadipocytes) were separated from mature adipocytes (MA, floating cells) for pre-treatment culture.

➢**Cell Culture:**

❑SVF Attachment cultures: SVF were cultured in triplicate for 24 hr in attachment media [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.

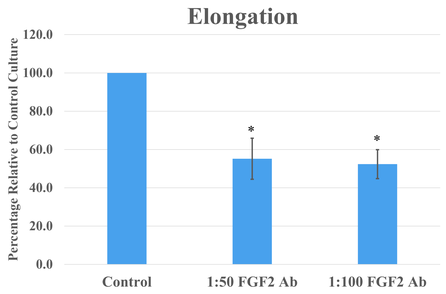
METHODS, cont.

○**MA Acclimation culture**: MA were cultured in triplicate in standard non-attachment media [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 for 24 hr to acclimate cells to culture.

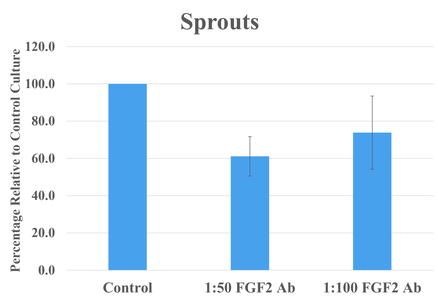
❑**Treatment Cultures**:

○Attachment media was removed from SVF cultures and replaced with standard media. MA cells were added to SVF cultures with and without treatment. Treatments were 1.) 0M (control, media), 2.) 1:50 dilution of FGF2 polyclonal antibody or 3) 1:100 FGF2 polyclonal antibody. Antibody treatment was utilized to inhibit endogenous FGF2 to confirm that MA influenced angiogenesis through FGF2. Cells were incubated for 24 hr under the same incubation conditions. After 24 hr time period, the cells were collected and terminated with denaturing solution. Angiogenic progression was documented via microscope.

RESULTS

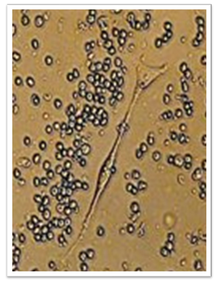


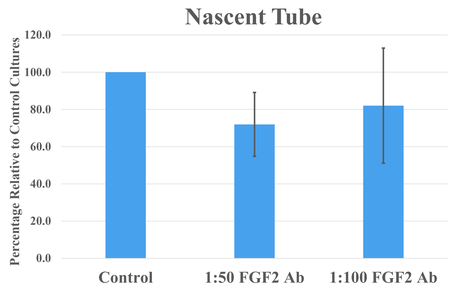
Elongation: Blocking endogenous FGF2 with FGF2 polyclonal antibody (FGF2 Ab; 1:50 or 1:100 dilution) decreased (*P=0.01) the number of VEC elongating relative to control.



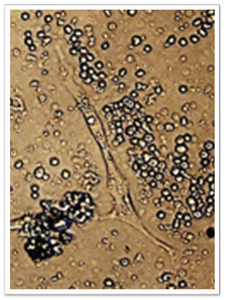
Sprouts: Blocking endogenous FGF2 with FGF2 polyclonal antibody (FGF2 Ab; 1:50 or 1:100 dilution) tended to decrease (*P=0.1) the number of VEC sprouting relative to control.

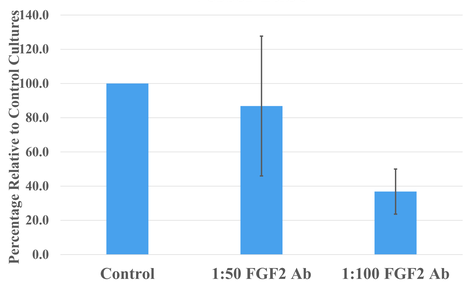
RESULTS, cont.





Nascent Tube: Blocking endogenous FGF2 with FGF2 polyclonal antibody (FGF2 Ab; 1:50 or 1:100 dilution) did not significantly influence VEC nascent tube formation relative to control.





Vessel Tube: Blocking endogenous FGF2 with FGF2 polyclonal antibody (FGF2 Ab; 1:50 or 1:100 dilution) did not significantly influence VEC vessel tube formation relative to control.

CONCLUSIONS

In conclusion, preliminary data demonstrated that endogenous FGF2 influences the early stages of morphologic progression of VEC in vessel tube formation. Although the later stages of morphological progression were not significant, numerical differences imply that FGF2 may be involved in tube formation, albeit, not to the extent of the earlier stages.

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