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Emily Leboffe
Susquehanna University

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The Effect of Matrix Metalloproteinase Inhibition on Intracellular Signaling in Adipogenic Differentiation of 3T3-L1 Cells

Emily Leboffe and Dr. Thomas Peeler
Department of Biology, Susquehanna University, Selinsgrove, PA

Abstract

The growing obesity epidemic has led to an increased interest in the study of adipogenesis, or fat cell development. Obesity has been shown to increase the risk of type 2 diabetes, high blood pressure, and a number of other diseases. 3T3-L1 fibroblast cells are a key model system to study the signaling events in the differentiation of preadipocytes into mature adipocytes. Extracellular matrix remodeling is a critical process during adipogenesis and is facilitated by matrix metalloproteinases (MMPs). Despite the known importance of extracellular matrix signaling, much of these signaling pathways are not well understood in adipogenesis. MMP inhibition has been shown to inhibit adipogenesis and be correlated with a decrease in the peroxisome proliferator-activated receptor γ (PPARγ). To better understand the importance of MMPs in adipogenesis, the MMP inhibitor ilomostat was used on mouse 3T3-L1 preadipocytes in addition to the PPARγ agonist rosiglitazone. We predicted that if MMP inhibition prevents activation of the transcription factor CCAAT/enhancer-binding protein β (CEBPβ), the effect of ilomostat should overcome the upregulation of downstream PPARγ by rosiglitazone. Results suggest that treatment with the inhibitor prevents adipogenic conversion despite the addition of the PPARγ agonist. Western Blot analysis showed that PPARγ expression was slightly upregulated in cells treated with rosiglitazone, and decreased in cells treated with both. The results of this study will help clarify the downstream effects of MMP signaling during adipogenesis and provide insight into specific targets for obesity treatment.

Methods

Maintenance of 3T3-L1 Cell Line. 3T3-L1 cells (American Type Culture Collection) were maintained in T-25 flasks in 10% fetal cord serum (FCS)/DMEM media (Dulbecco’s Modified Eagle Media with 10% donor bovine serum, 1% penicillin/streptomycin, 1% sodium pyruvate, and 1% L-glutamine). Cells were incubated at 37°C with 10% CO2. Media was changed every two days until 70% confluence. Cells were plated in 6-well plates in fresh media until 100% confluence. Two days post confluence (Day 0), the differentiation media was added. 10% FBS/DMEM media with 1% insulin, 1,000 isouleomethylenes, 1,000 deoxymethasone, and 1,000 insulin. On Day 2 the media was replaced with 10% FBS/DMEM media with 1,000 insulin. On Day 4, the media was replaced with only 10% FBS/DMEM media. Every two days thereafter the media was changed to fresh differentiation media, until Day 8. Treatment Protocols. Cells were treated with rosiglitazone at 2 µM, added on Day 0 of treatment and incubated with the cells for two days. Ilomostat was added at a concentration of 25 µM, added on Day 0 and Day 6 over two days through differentiation. Differentiation and growth media controls received 40 µg/mL DMEM. Oil Red O Staining. Oil Red O Staining was used to measure lipid accumulation on Day 8. Pictures of the cells in the well were taken to observe lipid accumulation. Once the stains were extracted, the absorbance of diluted solutions were measured at 530 nm.

Western Blotting: Protein Extraction. At desired time points in the differentiation, media was removed and the cells were washed with 1x PBS, and then suspended in 100 µL of Laemmli buffer. The samples were frozen and stored at -20°C.

Gel Electrophoresis and Protein Transfer. Frozen protein samples were prepared by heating for three minutes at 95°C. Proteins were separated by 10% SDS-polyacrylamide gel (BioRad). Proteins were transferred and immobilized on a nitrocellulose membrane at 4°C.

Blotting. The membranes were blocked in 5% non-fat milk in TBST at room temperature for one hour. Membranes were incubated at 4°C in an anti-MMP purified rabbit monoclonal antibody (abcam) 1:1000. Membranes were incubated in secondary antibody for one hour at room temperature in darkness anti-rabbit HRP antibody (abcam) 1:3000. Membranes were imaged using an ImageQuant LAS 4000.

Intracellular Signaling

Figure 1: Intracellular signaling events in adipogenesis. Control of adipogenesis is dependent on regulation of PPARα and CEBP family transcription factors. PPARα is the master regulator of adipogenesis, but it is dependent on activation by CEBP transcription factors.

Extracellular Matrix Remodeling

Figure 2: Extracellular matrix remodeling is a critical adipogenesis process. Preadipocyte ECM is supported by type 1 and type IV collagen fibers and a fibronectin network. MMPs in cell membrane class integrin attachments to fibronectin during adipogenesis facilitating the switch to a lamina network in mature adipocytes.

Adipogenesis Visualization

Figure 3: Model of ilomostat/rosiglitazone signaling interaction. MMP inhibition with ilomostat may be correlated with a decrease in CEBPβ DNA binding capacity (Croissandeau et al., 2002), which leads to reduced transcriptional activation of PPARγ, and would decrease the effect of rosiglitazone. Details on the signaling events between MMP function and intracellular signals is not well understood.

Figure 4: Cell imaging of treatment cells on Day 8. (A) After Oil Red O staining, the red color indicates lipid accumulation in cells. (B) Cells imaged with a light microscope show lipid droplets in cells that underwent adipogenesis. In both A and B cells treated with just rosiglitazone have increased lipid than the controls. Cells treated with rosiglitazone and ilomostat have decreased lipid than both controls and rosiglitazone alone. This indicates ilomostat can overcome the effect of rosiglitazone, suggesting that inhibiting MMPs can overcome PPARγ upregulation.

PPARγ Expression

Figure 5: Oil Red O staining on Day 8 of treatment. Samples (n=6) were measured at 530 nm to measure lipid accumulation in cells, with higher lipid accumulation indicating more cells that underwent adipogenesis. Values were normalized to the differentiation media control for comparison of trials. Cells treated with only rosiglitazone showed the highest level of lipid. Cells treated with ilomostat showed lipid levels lower than both rosiglitazone and the differentiation control. Indicating ilomostat overcome the upregulation of PPARγ by rosiglitazone on the cells.

Conclusions

- The effect of ilomostat cannot be rescued by treatment with rosiglitazone
- MMP inhibition prevents adipogenesis despite the addition of a PPARγ agonist
- Rosiglitazone functions if PPARγ protein is present, therefore the decrease in PPARγ expression may be from decreasing PPARγ transcription
- This study supports the proposal that MMP inhibition decreases the ability of C/EBPβ to bind DNA and induce PPARγ transcription

Future Directions

- Measure C/EBPβ expression after treatments with rosiglitazone and ilomostat
- Inhibit specific MMPs in the membrane and study their effect on adipogenic signaling
- Clarification of the downstream effects of MMP signaling during adipogenesis will provide more insight into specific targets for obesity treatment

References


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