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Title: An Adipocyte Cell Culture Model to Study the Impact of Protein and Micro-RNA Modulation on Adipocyte Function

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Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **NO**

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **NO**

3. **Interview statements:** Considering the COVID-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one**.

   ☒ Interviewees wear masks until videographer steps away (≥6 ft/2 m) and begins filming, then the interviewee removes the mask for line delivery only. When take is captured, the interviewee puts the mask back on. Statements can be filmed outside if weather permits.

4. **Filming location:** Will the filming need to take place in multiple locations? **NO**

Current Protocol Length

Number of Steps: 16
Number of Shots: 34

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Jennifer Jager:** This protocol makes it possible to manipulate protein or micro-RNA expression in differentiated adipocytes to study their role in adipocyte function.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

1.2. **Jennifer Jager:** This reverse transfection method is a simple, economical, and highly efficient method to transfect oligonucleotides into mouse 3T3-L1 adipocytes.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

OPTIONAL:

1.3. **Jennifer Jager:** This method can also be applied to human preadipocytes differentiated into adipocytes.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Introduction of Demonstrator on Camera

1.4. **Jennifer Jager:** Demonstrating the procedure will be Mélanie Gaudfrin, an engineer/technician from my laboratory.

1.4.1. INTERVIEW: Author saying the above.

1.4.2. The named demonstrator(s) looks up from workbench or desk or microscope and acknowledges the camera.

Protocol

2. Differentiation of murine 3T3-L1 fibroblasts into adipocytes and preparation of precoated plates

2.1. Grow the 3T3-L1 fibroblasts in 100-millimeter dishes in DMEM without pyruvate, 25 millimolar glucose, 10% newborn calf serum, and 1% penicillin and streptomycin **[1-TXT]**. Place the dishes in a tissue culture incubator at 37 degrees Celsius and 7% carbon dioxide **[2]**.

2.1.1. Talent inoculating 3T3-L1 fibroblast cells in dishes containing DMEM medium. **TEXT: DMEM-Dulbecco's minimum essential medium**

2.1.2. Talent placing the dishes in the incubator.

2.2. Two days after confluence, change the culture medium, replacing it with DMEM without pyruvate, 25 millimolar glucose, 10% FCS, and 1% penicillin and streptomycin supplemented with 0.25 millimolar IBMX, 0.25 micromolar dexamethasone, 5 micrograms per milliliter insulin, and 10 micromolar rosiglitazone **[1-TXT]**. Incubate the dishes for 2 days **[2].**

2.2.1. Talent changing the medium. **TEXT: IBMX-3-Isobutyl-1-methylxanthine**

2.2.2. Talent incubating the dishes in the incubator.

2.3. Two days later, replace the culture medium with DMEM without pyruvate, 25 millimolar glucose, 10% FCS, and 1% penicillin and streptomycin supplemented with 5 micrograms per milliliter insulin and 10 micromolar rosiglitazone **[1]** and incubate for another 2 days **[2]**.

2.3.1. Talent changing the medium.

2.3.2. Talent incubating the dishes in the incubator.

2.4. Feed the cells every 2 days with DMEM without pyruvate, 25 millimolar glucose, 10% FCS, and 1% penicillin and streptomycin **[1]** and keep the cells in the incubator **[2]**.

2.4.1. Talent feeding cells with medium.

2.4.2. Talent incubating the dishes in the incubator.

2.5. One day or a few hours before the transfection, prepare a solution of collagen type I *('one')* at 100 micrograms per milliliter in 30% ethanol from a stock solution at 1 milligram per milliliter **[1]**.

2.5.1. Talent preparing the collagen type I solution.

2.6. Add 250 microliters of collagen per well of a 12-well plate or 125 microliters per well of a 24-well plate **[1]** and spread the solution over the surface of the well **[2]**. *Videographer: This step is important!*

2.6.1. Talent adding the collagen solution in the 12- and 24-well plates.

2.6.2. Talent spreading the solution over the surface of the well.

2.7. Leave the plate without the lid under the culture hood until the collagen dries **[1]**, then wash it twice with D-PBS **[2-TXT]**.

2.7.1. Talent drying the collagen solution.

2.7.2. Talent washing the plate with D-PBS. **TEXT: D-PBS- Dulbecco's phosphate-buffered saline**

NOTE: Action in shot 2.7.2 was changed during shoot.

3. Preparation of the transfection mix and 3T3-L1 adipocytes

3.1. Pipette the siRNA *(spell out)* with improved Minimum Essential Medium to mix **[1-TXT]** and incubate for 5 minutes at room temperature **[2]**.

3.1.1. Talent mixing the siRNA with medium using pipette. **TEXT: siRNA- small-interfering RNA**

3.1.2. Talent incubating the siRNA at room temperature.

3.2. Add the transfection reagent and the improved Minimal Essential Medium to the siRNA **[1]** and pipette to mix **[2]**. Incubate it for 20 minutes at room temperature **[3]**, then add the transfection mix to each well of the collagen-coated plate **[4]**.

3.2.1. Talent adding the transfection reagent and the Minimal Essential Medium to the siRNA.

NOTE: Additional action was performed in shot 3.2.1 during shoot.

3.2.2. Talent mixing the reagent.

3.2.3. Talent incubating the reagent at room temperature.

3.2.4. Talent adding this transfection mix solution to each well of collagen-coated plate.

3.3. Wash the cells in the 100-millimeter Petri dish twice with D-PBS **[1]**. Add 5x trypsin to the cells, making sure to cover the entire surface with the trypsin **[2-TXT]**. Wait for 30 seconds **[3]** and carefully remove the trypsin **[4]**.

3.3.1. Talent washing the cells with D-PBS.

3.3.2. Talent adding 5x trypsin to the cells. **TEXT: 1 mL per 100 mm dish**

3.3.3. Talent incubating the cells in trypsin for 30 seconds.

3.3.4. Talent removing the trypsin.

3.4. Incubate the Petri dish for 5 to 10 minutes at 37 degrees Celsius in the incubator **[1]**. Then, tap the dish to detach the cells avoiding cell damage **[2]**. *Videographer: This step is difficult and important!*

3.4.1. Talent incubating the dish in incubator.

3.4.2. Talent tapping the dish.

3.5. Add 10 milliliters of DMEM without pyruvate, 25 millimolar glucose, 10% FCS, and 1% penicillin and streptomycin to neutralize the trypsin **[1]**. Carefully pipette the medium up and down to detach the cells and homogenize the cell suspension **[2]**. *Videographer: This step is difficult and important!*

3.5.1. Talent adding DMEM medium in to the cells.

3.5.2. Talent pipetting the medium up and down.

3.6. Count the cells using a Malassez counting chamber or an automated cell counter **[1]**, and adjust the concentration of the cells to 0.625 million cells per milliliter of medium **[2]**.

3.6.1. Talent counting the cells.

3.6.2. Talent adjusting the concentration of the cells using medium.

3.7. Seed 800 microliters of the cell suspension per well of a 12-well plate or 400 microliters of the cell suspension per well of a 24-well plate containing the transfection mix **[1-TXT]**.

3.7.1. Talent adding cells in to the plates. **TEXT: 5 x 10^5 cells/well for 12-well plate and 2.5 x 10^5 cells/well for 24-well plate**

3.8. Incubate the plates in a cell culture incubator and do not disturb them for 24 hours **[1]**. On the next day, carefully replace the supernatant with fresh DMEM without pyruvate, 25 millimolar glucose, 10% FCS, and 1% penicillin and streptomycin **[2]** and return the cells to the incubator **[3]**.

3.8.1. Talent incubating the plates in incubator.

3.8.2. Talent replacing the medium next day.

3.8.3. Talent incubating the dish in incubator.

3.9. Study target knockdown 24 to 48 hours and 48 to 96 hours after siRNA or miR *(spell out)* mimic delivery for mRNA *(spell out)* and protein, respectively. Perform functional analyses of transfected adipocytes to study insulin signaling, glucose uptake, adipokine secretion, lipolysis, and lipogenesis **[1]**.

3.9.1. Talent looking at analysis results on a computer.

Results

4. Differentiation, protein silencing, micro-RNA overexpression, and effects of protein or micro-RNA modulation in 3T3-L1 adipocytes

4.1. The adipocytes have been shown to preserve their morphology after the transfection **[1]**. Two days after transfection, the adipocytes presented multilocular lipid droplets with lipid content not different between the transfected and non-transfected adipocytes **[2]**.

4.1.1. LAB MEDIA: Figure 1B and 1C.

4.1.2. LAB MEDIA: Figure 1D and 1E.

4.2. The mRNA expression of various differentiation markers was unchanged in transfected cells compared to that in non-transfected adipocytes **[1]**.

4.2.1. LAB MEDIA: Figure 1F.

4.3. The reverse-transfection protocol is efficient as more than 70% of the adipocytes were transfected **[1]**.

4.3.1. LAB MEDIA: Figure 1G and 1H.

4.4. Three days after the transfection with siRNA *(spell out)* against *Plin1 ('perilipin one')*, the mRNA level of *Plin1* had decreased by 70% **[1]** and the protein level by 63% **[2]**.

4.4.1. LAB MEDIA: Figure 2A.

4.4.2. LAB MEDIA: Figure 2B and 2C.

4.5. PLIN1 expression was also analyzed by fluorescence microscopy 4 days after the transfection and was found to have decreased by 92% compared to its expression in control adipocytes **[1]**.

4.5.1. LAB MEDIA: Figure 2D, 2E, and 2F. *Video editor focus on the pink spots in Figure 2D and 2E.*

4.6. The reverse-transfection of adipocytes with micro-RNA mimicking oligonucleotides to upregulate the expression of miR-34a is shown here **[1]**. The overexpression of miR-34a led to the decrease in VAMP2 *('vamp-2')* protein expression by 50% **[2]**.

4.6.1. LAB MEDIA: Figure 3A.

4.6.2. LAB MEDIA: Figure 3B and 3C.

4.7. The knockdown of *Plin1* in 3T3-L1 adipocytes led to an increase in basal lipolysis **[1]**, while the overexpression of miR-34a led to the inhibition of insulin-induced protein kinase B phosphorylation **[2]** and glucose uptake **[3]**.

4.7.1. LAB MEDIA: Figure 4A.

4.7.2. LAB MEDIA: Figure 4B and 4C.

4.7.3. LAB MEDIA: Figure 4D.

Conclusion

5. Conclusion Interview Statements

5.1. **Jennifer Jager:** It is important to reach a high level of adipocyte differentiation, to perform the transfection on newly differentiated adipocytes, and to carefully monitor the treatment of adipocytes with trypsin.

5.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.2, 2.3, and 3.3*

5.2. **Jennifer Jager:** Following the transfection it is possible to perform functional experiments on the adipocytes to study the impact of proteins or micro-RNA manipulation on insulin signaling, glucose uptake, lipogenesis and lipolysis.

5.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.