

## **Modulation of Protein Expression in Isolated Pancreatic Acini Using Adenoviral or Lentiviral Vectors**

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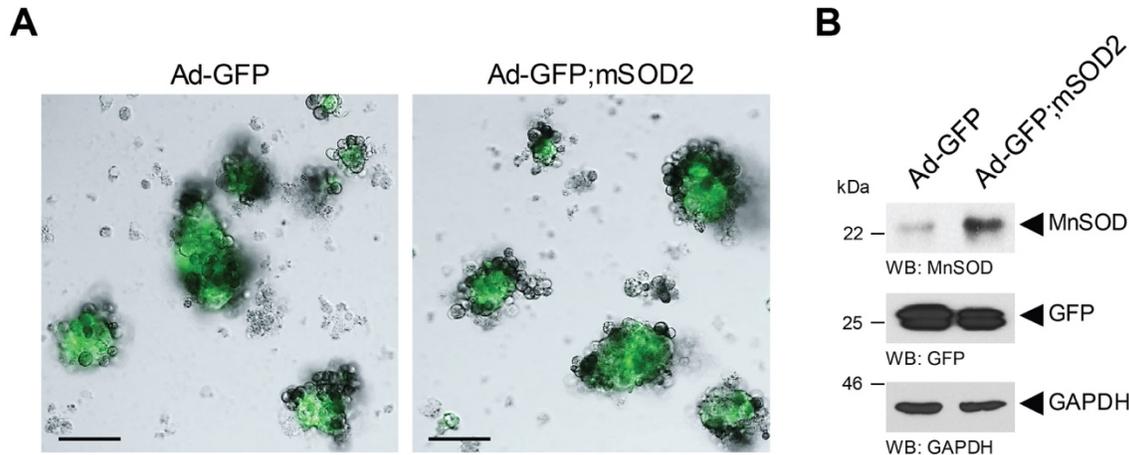
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### **1. Introduction**

Acinar cells represent the exocrine part of the pancreatic gland, where large amounts of digestive enzymes are produced, stored and secreted. These processes are regulated by external and internal signaling events and disruption of these normal functions can lead to chronic diseases. For example, due to the high rate of protein synthesis, acinar cells show increased levels of endoplasmic reticulum (ER) stress, which can be compensated for under normal conditions. A failure of such compensation leads to acinar cell destruction, pancreatic inflammation and disease (8). In addition to this, acinar cells show high plasticity (15, 16), where stress and growth factor signaling can initiate their de- or transdifferentiation to a progenitor-like phenotype that is highly proliferative (12). This process is termed acinar-to-ductal metaplasia (ADM). ADM is observed *in vivo* during pancreatitis (7), where it is a reversible process and contributes to acinar cell regeneration after the insult (17). Irreversible ADM is observed after acquisition of an oncogenic KRas mutation (1, 6, 18) and is an initiating event for the development of pancreatic intraepithelial neoplasia (PanIN), precursor lesions for pancreatic ductal adenocarcinoma (PDA).

The assessment of signaling pathways that regulate normal acinar cell functions, such as

amylase secretion, or processes that drive acinar cell metaplasia and proliferation, often requires depletion of genes of interest using RNAi techniques or ectopic expression of wildtype or modified proteins. While primary acinar cells are difficult to transfect with common lipofection methods, lenti- or adenoviral delivery systems are most efficient (3, 4, 14). Lentiviral or adenoviral delivery methods have been used for multiple applications, such as ectopic expression of proteins (3, 6, 10, 13, 14, 19, 20, 21), knockdown of protein expression using shRNA (4, 11), expression of cre-recombinase to induce expression of transgene alleles (5), or for introduction of gene reporters (5). Moreover, a combination of lentiviral and adenoviral systems allows the knockdown and overexpression of genes of interest in the same cells. For example, using such a combination approach, it was shown that inhibitory effects on the ADM process due to lentiviral knockdown of endogenous PKD1 could be rescued by adenoviral overexpression of an allele of PKD1 from another species (4). In addition, the use of polycistronic vectors allows the expression of the mRNA or shRNA of interest as well as a reporter (such as GFP) to monitor infection efficiency (**Fig. 1A**). Here, we outline the procedure to modulate genes of interest in isolated primary acinar cells from mouse pancreas using adenoviral or lentiviral expression systems.



**Fig.1. Adenoviral infection and analyses of mouse primary acinar cells.** Primary acini isolated from mouse pancreas were infected with adenovirus harboring GFP (control) or GFP and SOD2 cDNA driven by a separate CMV promoter according to the procedures delineated above. **A:** 48 hours post infection, cells were analyzed for expression of GFP using an Olympus IX81 spinning disk confocal microscope (10x magnification; shown is an overlay of GFP and bright field). The bar indicates 100  $\mu\text{m}$ . **B:** Cells were lysed and analyzed by Western blot for GFP, SOD2 and GAPDH expression using anti-GFP (Santa Cruz, SC-9996, 1:2000) and anti-MnSOD (Abcam, ab68155, 1:2000) antibodies. Staining for GAPDH (anti-GAPDH, Cell Signaling, #5174, 1:1000) served as a loading control. Methods for generation of lysates and Western blotting are described in detail in (4, 5).

## 2. Materials

### 2.1 Culture Media and Reagents

1. Waymouth complete media (Waymouth media supplemented with 1% FBS, 0.1 mg/mL trypsin inhibitor and 1  $\mu\text{g}/\text{mL}$  dexamethasone)
2. For lentiviral infections: Polybrene solution (stock 6 mg/mL). Avoid freezing/thawing the polybrene working stock solution more than three times to ensure its activity.
3. Solution containing adenovirus or lentivirus carrying the gene of interest or empty vector (e.g. Ad-null) or carrying shRNA that targets the gene of interest or non-target shRNA (control) (**Note 1**).

### 2.2 Other materials

1. Low adhesive plastic dishes (e.g. Ultra-Low Attachment multiwell plates from Corning)

2. Biohazardous container to transport virus solutions between  $-80^{\circ}\text{C}$  freezer and cell culture room/hood
3. Barrier tips
4. 15 mL sterile plastic tubes (cell culture level)

## 3. Methods

1. Safety
  - a. Safe handling of viral solutions and media containing virus requires wearing person protection equipment (PPE) such as a lab coat, nitrile gloves, and safety goggles.
  - b. For decontamination purposes, all tips used for the infection procedure should be soaked in 10% bleach for at least 15 minutes. The cell culture hood, pipettes and other equipment should be sprayed with 10% bleach, left for at least 15

minutes, and finally cleaned with 70% ethanol.

2. Generation of adeno- or lentivirus particles and determination of titer.

- a. To generate adeno- or lentivirus, the gene of interest or shRNA can be cloned into commercially-available adenoviral or lentiviral expression vectors (e.g. ViraPower™ Adenovirus expression system, Invitrogen, Carlsbad, CA). Alternatively (recommended), there are multiple companies offering prepared adenovirus or lentivirus particles or generation of plasmids either for overexpression of genes of interest (i.e. OriGene, Rockville, MD or Vector Biolabs, Malvern, PA) or for shRNA expression (i.e. MISSION® shRNA libraries from Sigma-Aldrich, St. Louis, MO, or Vector Biolabs). For generation of adenovirus particles from existing plasmids, amplify and purify adenovirus from HEK293 cells using Adeno-XTM maxi purification kit from Takara Bio (Mountain View, CA) according to the manufacturer's instructions. For generation of lentivirus particles, co-transfect HEK293FT cells of low passage with a lentiviral plasmid and viral packaging mix (i.e. ViraPower™ packaging mix from Invitrogen) according to the manufacturer's instructions.
- b. For lentiviral knockdown, we usually obtain a set of five different shRNA virus constructs from Sigma-Aldrich and then test which two sequences are most efficient in knocking-down the target gene. This evaluation is usually performed by co-transfecting the shRNA construct together with an expression plasmid that allows ectopic expression of a tagged version of the protein of interest into an easy to

transfect mammalian cell line (e.g. HeLa). The two best shRNA sequences are then used for lentivirus generation and titer determination. The comparison of effects obtained with two different shRNA sequences will allow exclusion of off-target effects.

- c. Adenoviral titers can be determined by multiple methods including detection of adenoviral particles and formation of plaques. We use the Adeno-XTM rapid titer kit from Takara Bio, which measures the production of adenovirus hexon protein in HEK293 cells. Lentiviral titers can be determined using a colony formation assay, in which HeLa cells are infected with different amounts of lentiviral solution at 10-fold serial dilutions. The infected cells are then selected for using the selection marker of the lentiviral construct (e.g. puromycin) for 10-14 days. Numbers of colonies formed are counted to obtain the lentiviral titer (transduction unit/mL). Viral titers may decrease with long-term storage at -80°C. Therefore, it is necessary to re-determine the viral titers when stock solutions are older than one year.
3. Isolate pancreatic acini from a mouse pancreas [method described in detail in (7) and also in (2, 9, 19, 20)] and re-suspend them in Waymouth complete media. Per experimental condition, seed 2 mL cell suspension on a low adhesive plastic dish.
  4. For lentiviral infections only: Add 2 µL polybrene stock solution to the pancreatic acini for each plate, and gently shake the plastic plate to ensure that polybrene is uniformly distributed. The final concentration of polybrene is 6 µg/mL.

5. Place acini into a humidified cell culture incubator (37°C, 5% CO<sub>2</sub>).
6. Retrieve solutions with adenovirus or lentivirus particles from -80°C freezer, and place on ice.
7. Defrost virus solutions by placing them in a 37°C water bath. Once the solutions are thawed, immediately take them out of the water bath. Before placing virus solutions into the tissue culture hood, sterilize the outside of the tubes/containers by spraying with 70% ethanol.
8. Remove the acinar cells/acini from the cell culture incubator and place them inside the cell culture hood.
9. Apply virus solutions to cells and then immediately put leftover viral solutions on ice, followed by storage at -80°C for future use. To avoid the decrease of viral titers, do not freeze/thaw the viral solutions more than three times. The final functional titer for adenovirus should be ~10<sup>7</sup> infectious units/mL (ifu/mL), or ~10<sup>7</sup> transduction units/mL (TU/mL) for lentivirus (**Note 2**).
10. Gently shake the low adhesive plastic dishes to ensure that the virus is uniformly distributed, and place them back into a humidified cell culture incubator.
11. For adenovirus, after 3 hours of infection, transfer the acini to a 15 mL tube and spin down cells at 1000 rpm for 2 min at RT, before plating for experiments (alternatively cells can be plated without washing off the virus). For lentiviral infections, spin down cells after 24 hours of infection and continue as above (for ADM assays or 3D organoid culture of infected cells, see **Note 3**).
12. Sterilize the outside of the 15 mL tubes that contain the mixture of pancreatic acini (pellet) and virus (supernatant) by spraying them with 70% ethanol.
13. Carefully move the acinar cells back to the cell culture hood without disturbing the cell pellets.
14. Remove supernatants and re-suspend pancreatic acinar cells/cell clusters in 2 mL of fresh Waymouth complete media.
15. Seed infected pancreatic acini on low adhesive plastic plates and put them back into a cell culture incubator.
16. Dependent on the viral delivery system used, approximately 24 to 72 hours post viral infection (**Note 4**), RNA or proteins can be extracted for analyses by quantitative real-time PCR or Western blotting.

#### 4. Notes

1. It should be kept in mind that an adenoviral delivery system requires less time for expression or knockdown of the gene of interest than lentiviral delivery systems. This may be of importance for analysis of isolated acinar cells. However, for the work with acinar cell clusters and analysis of ADM we did not observe a disadvantage of using lentiviral systems. In addition, the combination of adenoviral and lentiviral systems allows the expression or knockdown of genes of interest in the same cells.
2. A titer of 10<sup>7</sup> ifu/mL was arrived at empirically. Using GFP adenovirus at this titer, 80% of isolated acini were GFP positive 48 hours after infection.
3. If virally-infected acinar cells will be embedded in extracellular matrix (ECM), such as collagen or Matrigel®, for ADM assays or 3D organoid culture (detailed methods described in (7))

skip washing off the virus in 3.8 and skip steps 3.9 to 3.12. Acinar cells should be embedded in ECM 3 to 5 hours after infection with adeno- or lentivirus. Of note: If studying molecules that affect the ADM process, seed acini in 3D collagen because Matrigel® by itself induces ADM.

4. When using acinar cell clusters to study the ADM process, we did not observe acinar cells death within 3-5 days. However, when single acinar cells were used for studies of secretion, it was shown that after 24 hours secretion is

minimal, possibly due to acinar cell degeneration. The viability of cells at the endpoint of the experiments can be tested by live cell dyes such as Hoechst 33342.

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