

Oligofection of small interfering RNAs in senescent human fibroblasts

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Introduction

Cellular senescence is a barrier to unlimited cellular proliferation. Senescent cells are alive and metabolically active but are unable to further divide. Cellular senescence may be the consequence of a number of stressing stimuli such as telomere shortening, exposure to DNA damaging agents and the expression of activated oncogenes. Its study impinges on a number of important biological mechanisms related to organismal aging and tumor development. Therefore, the study of the pathways involved in its establishment, maintenance or escape is of interest for many scientists. Unfortunately, senescent cells are not amenable to the usual cellular techniques commonly used in molecular and cellular biology to modulate cellular factors: plasmids cannot be efficiently transfected by standard calcium precipitation or liposomes-based techniques and microinjection is hard due to the flattened morphology of senescent cells. Furthermore, their general little availability make them precious and suggest the use of approaches that minimize their use yet retain informative value. An approach can be the use of lentiviral vectors, recombinant viruses that can integrate and express also in non-dividing cells. The generation of lentiviruses expressing RNA interference transcripts against cellular genes has opened the possibility to knockdown individual genes in senescent cells. Nevertheless, not all molecular biology laboratories are equipped or have experience with lentivirus production.

An easier and equally efficient alternative is the transfection of short interfering RNAs (siRNAs) by lipofection. Differently from plasmid DNA, siRNAs can efficiently enter the cells and their transfection efficacy can be directly monitored by the use of fluorescent labeled RNA oligonucleotides.

We present here a brief method that allows the transfection of human senescent cells (regardless of the cause of cellular senescence) with an efficiency of around 70%.

Reagents

Oligofectamine (INVITROGEN cat. 12-252011)

Optimem (INVITROGEN cat. 51985-026)

Oligos (Dharmacon)

Poly-D-lysine (SIGMA cat.P6407 5mg)

Equipment

Coverslips

24-well plates

Procedure

- 1) Prepare poly-D-lysinated coverslips (poly-D-lysine 50 µg/ml)
- 2) Plate $15\text{-}20 \times 10^3$ cells per coverslip (12 mm diameter) 2 days before the oligofection.
- 3) Mix 175µl Optimem + 1-10µl siRNA oligos (200 nM) per coverslip.
- 4) Mix 11µl Optimem + 4µl Oligofectamine per coverslip and leave for 7 minutes.
- 5) Mix solution obtained in 2) and 3) and leave for 15 minutes.
- 6) During the incubation time, change the medium of senescent cells: remove old medium and add 800µl Optimem per coverslip.
- 7) Add mix obtained in 4) to senescent cells already in 800µl Optimem.
- 8) Leave cells in the incubator for at least 4 hours.
- 9) Without removing the Optimem medium, add 1ml of medium used to culture the senescent cells without antibiotics with 20% FCS or FBS.

Timing

4,30 h

Troubleshooting

Once resuspended, the siRNA oligos should be stored in small aliquots at -20°C . For best results, limit freeze-thaw cycles of each tube to no more than 5 cycles.

Ras expressing senescent cells are prone to detach, wash gently

Always use poly-D-lysinated coverslips.

References

d'Adda di Fagagna *et al.* A DNA damage checkpoint response in telomere-initiated senescence *Nature* **426**, 194-198 (2003)

Figures

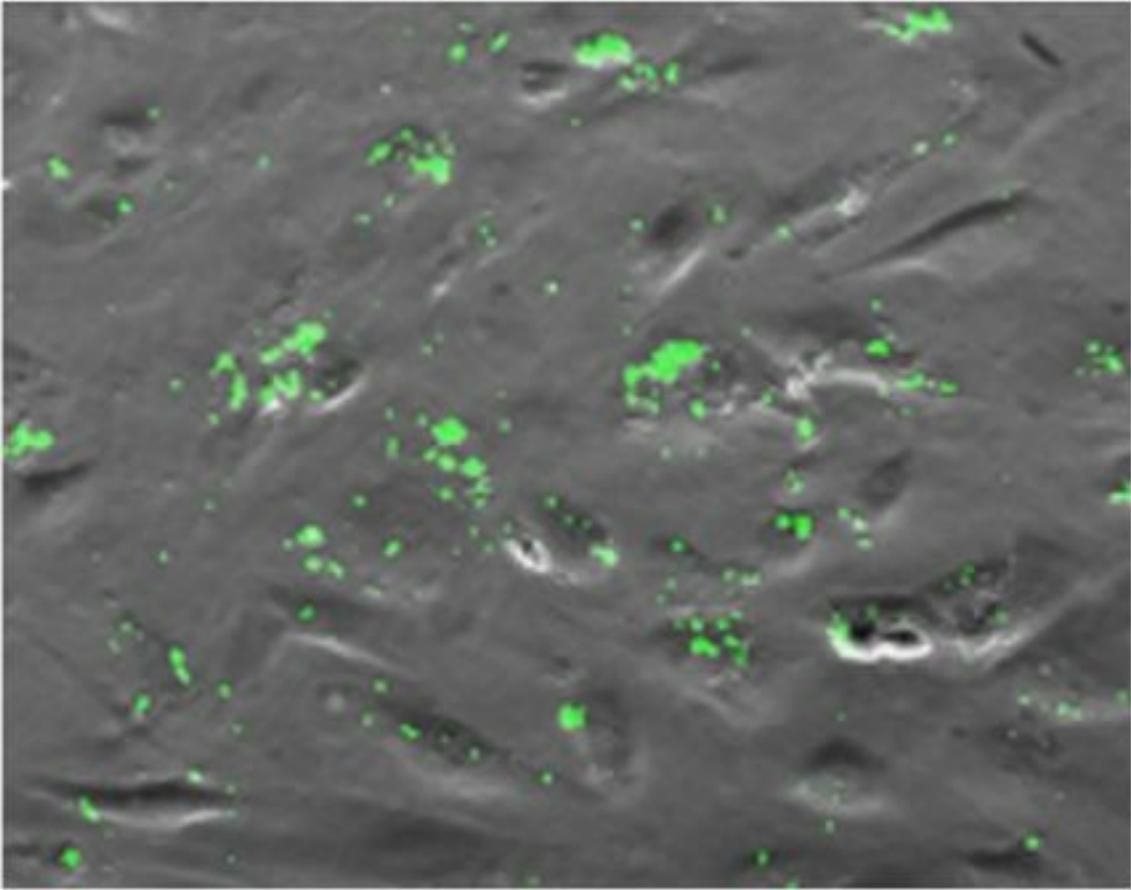


Figure 1

Oligofection efficiency Efficiency of oligofection: 70% of cells are transfected, as demonstrated by the internalization of a fluorescent-labeled double-stranded RNA oligonucleotide

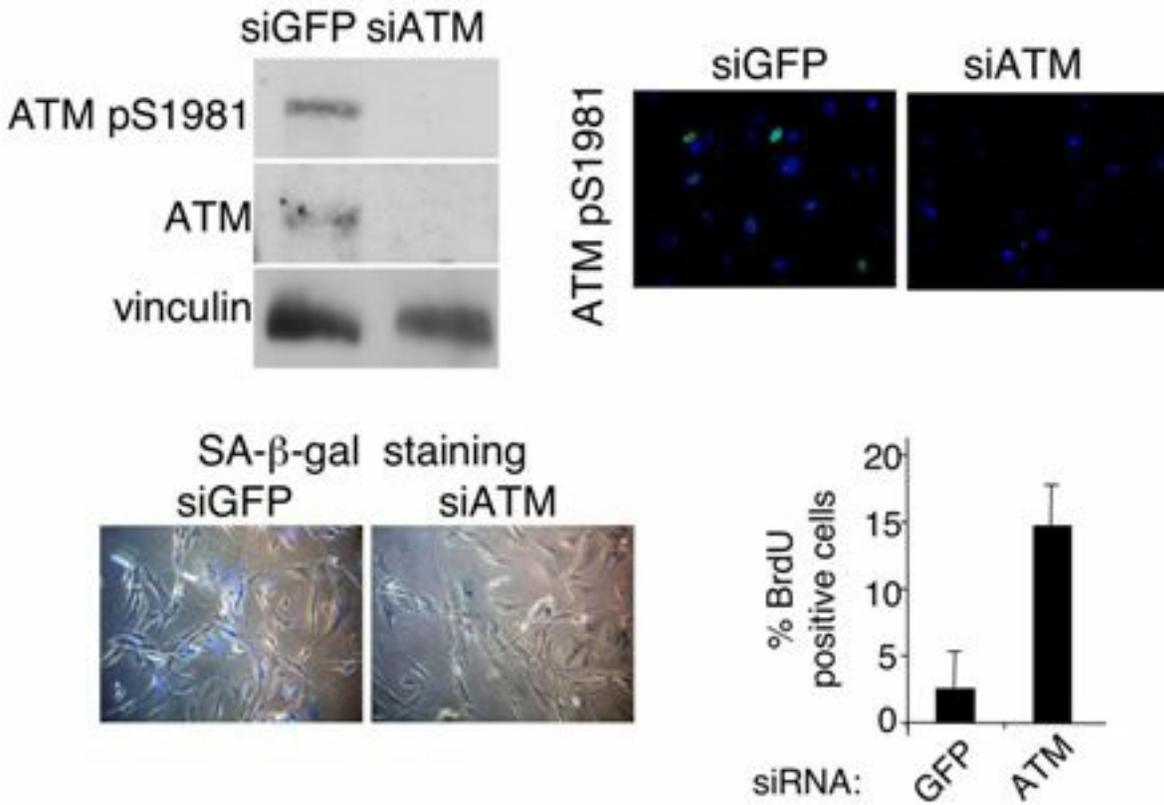


Figure 2

Example of DDR knockdown efficiency in oncogene-induced senescent cells Demonstration of DDR knockdown efficiency by transiently transfected siRNAs in oncogene induced senescent cells. OIS cells were transfected with the indicated siRNA (siATM) and probed by immunofluorescence or stained for senescence-associated β -galactosidase three days later. Cells were also lysed and extracts used for immunoblotting assays. DDR interfered cells re-enter S-phase of the cell cycle as shown by BrdU incorporation.

Oncogene-induced senescence is a DNA damage response triggered by DNA hyper-replication

by Di Micco, R. et al.

Nature (03 October, 2006)