Transfection of HaCaT, human immortalized keratinocytes with METAFECTENE- PRO.

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Introduction:

Delivery of DNA into primary or immortalized keratinocytes, without the help of viral systems, has proven to be a difficult task. HaCaT is a spontaneously immortalized human keratinocyte cell line that can be induced to differentiate by calcium and is widely used as a tool for studying gene function and regulation in keratinocytes. In litterature, very few transfection reagents were shown to be effective in HaCaT cells, moreover, when documented, transfection efficiency were largely unsatisfactory. In our laboratory we are investigating on the mechanisms regulating p63 protein stability. The p63 protein belongs to the tumor suppressor p53 gene family. The p63 locus encodes at least six different p63 isoforms but only one of them, ΔNp63α, is abundantly expressed in primary and immortalized human keratinocytes. So far, our studies on the role of p63 protein isoforms have been performed by transfecting p63 in cell lines such as H1299, Saos2 and U2OS, where p63 is not normally expressed (Calabrò V. et al. JBC 2002; Calabrò V. et al. MCB 2004; Lo Iacono et al. Cell Cycle 2006); however we wished to confirm our results in a more physiological context such as the HaCaT cells. The “Fugene” reagent which is the most widely used reagent to transfect HaCaT cells is quite expensive. In our hand, it also turned out to be unstable and not enough efficient in transfecting HaCaT cells. Thus, we have decided to try the novel reagent METAFECTENE-PRO from Biontex.

Materials and methods:

Metafectene PRO, a polycationic liposomal transfection reagent, was obtained from Biontex Laboratories GmbH (Munich, Germany). The plasmid pcDNA3-TAp63α was kindly provided by Dr. Hans Van Bokhoven. Human spontaneously immortalized HaCaT cells were kindly provided by Dr. Antonio Costanzo (University of Rome “Tor Vergata” Italy). HaCat cells were cultured in Dulbecco’s modified Eagle’s MEM (DMEM) supplemented with 10% FBS (EuroClone), penicillin (100 U/ml), streptomycin (100μg/ml) and L-glutamine (4mM).

Experimental procedures / transfection protocol:

For transfection, HaCaT (3.0 x10 ^{5} cells) were seeded in 2 ml of DMEM in 35mm culture plates one day before transfection. One hour before transfection the medium was replaced with fresh complete DMEM medium. DNA-Metafectene complexes were prepared by adding different amount of Metafectene-PRO diluted up to 100 μl

with serum-free medium to 100 μl serum free DMEM/plasmid DNA (2 μg) for a total of 200μl. The mixture (DNA + transfection reagent) was incubated for 20 min at room temperature. Metafectene-DNA complexes were diluted in a volume of 2 ml of serum-containing medium and added to the cells. Upon 4h at 37°C the medium was replaced. Cells were lysated in 10mM Tris-HCL (pH 7.5), 1 mM EDTA, 150 mM NaCl, 1mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 0,5% sodium deoxycholate, and protease inhibitors. The Bio-Rad protein Assay was used to determine protein concentration. 40μg of total lysates, were immunoblotted and revealed with anti-p63 4A4 antibody. A parallel experiment was performed with a 2 μg of pcDNA3 vector expressing the Green Fluorescent protein, in order to evaluate the transfection efficiency by counting GFP positive cells under a fluorescent microscope.

Results and discussion:

We have transfected HaCaT cells using a Metafectene-PRO/DNA ratio of 1:2, 1:3, 1:5, 1:6 (μg DNA/μl reagent). Transfection efficiency ranged from 10% to 25%. The maximum efficiency was obtained at a 1:6 ratio with a 25% GFP positive cells using 0.8 μg GFP plasmid + 1.2 μg carrier DNA. When compared to mock-transfected controls, Metafectene-PRO did not result in a reduction of total amounts of cellular extract. We have not observed toxic effects of Metafectene-PRO. As shown in figure 1, expression of TAp63α exogenous protein in 40 μl of total lysates was abundant especially in lane 5, corresponding to the cells transfected to a 1:6 DNA/reagent ratio.

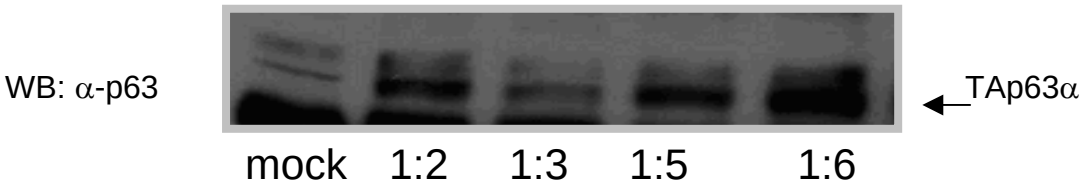
Conclusion / summary:

Metafectene-PRO is highly effective in transfecting human immortalized HaCat keratinocytes and it is considerably improved respect to the previous version of Metafectene that was completely uneffective on this cell line, at least in our experimental conditions. Toxicity of Metafectene-PRO was lower, when compared to the “Fugene” or Metafectene reagent.

References:

-Calabrò V., et al. “The human MDM2 oncoprotein increases the trascriptional activity and the protein level of the p53-homologue p63” . J Biol Chem. 2002 Jan 25;277(4):2674-81.
-Calabrò V., et al. (2004) Inhibition of p63 transcriptional activity by $p14^{ARF}$: functional and physical link between human ARF tumor suppressor and a member of the p53 family. Mol Cell Biol 24 (19), 8529-8540.
-Lo Iacono M., et al. (2006) The Hay Wells Syndrome derived TAp63Q540L mutant has impaired transcriptional and cell growth regulatory activity. Cell Cycle.

Appendix: Tables and/or Figures:



Western blot and specific immunodetection of transfected p63 protein in HaCat cells with Metafectene-PRO. The arrow indicates the band corresponding to the transfected TAp63 protein: 40 μg of total extract loaded. (TAp63 plasmid=0.8 μg + 1.2 μg of carrier DNA). Lane1:mock Lanes 2 to 5 transfected with the indicated ratio of DNA/Metafectene-PRO.