Transfection of a GFP expressing vector in transformed HCT 116 and HEP3B cells

Ferdinando Chiaradonna and Fabrizia Mastroianni

University of Milano Bicocca, Piazza della Scienza 2

20126 Milano

Introduction:

Autophagy is a complex catabolic program for lysosomal degradation of proteins and other subcellular constituents. It is part of everyday normal cell growth and development to maintain a balance between biogenesis (production) of cell structures, and their degradation and turnover. Nevertheless autophagy is often activated in response to nutrient deprivation for mantaining cell survival and differentiation (Dunn WA,Trends Cell Biol,1994). LC3 (rat microtubule-associated protein 1 light chain 3) is the first mammalian protein identified that specifically associates with autophagosome membrane after processing (Kabeya Y et al, EMBO J, 2000). We used the EGFP-C1 plasmid to test the transfection reagent (Metafectene-Pro, Biontex Laboratories). We than tranfected our cells with GFP-LC3 plasmid to evaluate the autophagic event in our experimental conditions by evaluating the cellular localization of LC3 protein (data not shown).

Materials and methods:

Cell lines: HCT 116 (human colorectal carcinoma)
HEP 3B (human hepatocellular carcinoma)

Transfected Plasmid: pEGFP-C1 and pEGFP-C1-LC3 (a gift of Dr. Y. Kabeya, Okazaki, Japan)

Tranfection reagent: Metafectene Pro, from Biontex Laboratories Gmbh (Munich, Germany)

Cells culture:

Human HCT 116 were grown in Dulbecco's Modified Eagle's Medium (Invitrogen, Carlsbad, CA, USA) containing 10% newborn calf serum, 4mM glutamine, 100U/ml penicillin, 100mg/ml streptomycin and 2% HEPES. HEP 3B cells were grown in Dulbecco's Modified Eagle's Medium containing 10% fetal bovin serum, 4mM glutamine, 100U/ml penicillin, 100mg/ml streptomycin and 1% HEPES. The cells were maintained at 37°C in a humidified atmosphere of 5% CO_2.

Experimental procedures / transfection protocol:

Cells were plated (1,5 x 10^5 cells/well) in 6-well plates containing 3 glasses/well. After 48h the cells were transfected with 1µg of DNA and different quantities of Metafectene Pro (2, 4, 6 µl). The Metafectene Pro in 50 µl of serum- and antibiotic-free medium was complexed with DNA diluted in 50 µl of serum- and antibiotic-free medium. The complexes were maintained 15 min at room temperature, then was added to cells and incubated at 37°C for 5h.
The reaction was stopped by replacing the culture medium. 24h after transfection images were taken with a Nikon microscope.

Results and discussion:

For HCT 116 cells: we obtained the better transfection efficiency with 4µl of Metafectene Pro.

For HEP 3B cells: we obtained the better transfection efficiency with 6µl of Metafectene Pro.

The same cell lines were transfected with calcium phosphate precipitation, but the result was worse than Metafectene Pro reagent.

We also transfected the NIH3T3 cells as described by Spain C. et al (www.biontex.it) with some modification. 2.5X10^5 cells/well were plated in 12-well culture plates and after 24h were transfected with 1µg of DNA and 2 µl of Metafectene Pro for 5h, but in this case we obtained a very low efficiency of transfection. To improve the efficiency of transfection we modified the above procedure. Specifically we plated 1x10^5 cells/well in 12-well and we transfected using 0,5µg and 1µg of DNA with two different amount of metafectene Pro (2-4 µl). The Metafectene Pro was used for 5h.

The best condition of transfection was obtained using 1µg of DNA and 4 µl of Metafectene Pro (data not shown).

Conclusion / summary:

We tested the Metafectene Pro reagent and calcium phosphate precipitation for transfection of pEGFP-C1 plasmid and we obtained better transfection efficiency with Metafectene Pro reagent; anyway the efficiency is dependent on cell type.

References:

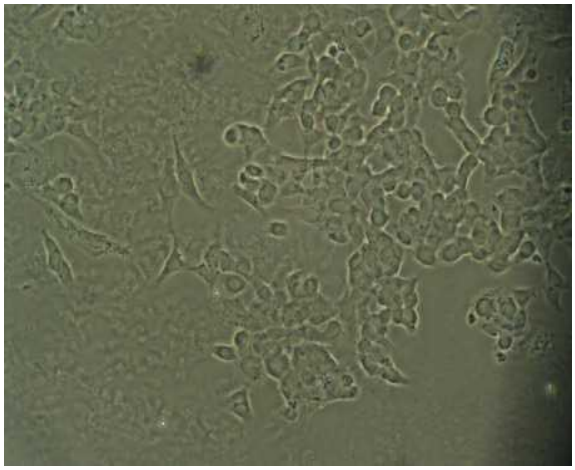
Dunn, W.A. (1994) Autophagy and related mechanism of lysosome-mediated protein degradation. *Trends Cell Biol*; 4:139-143.

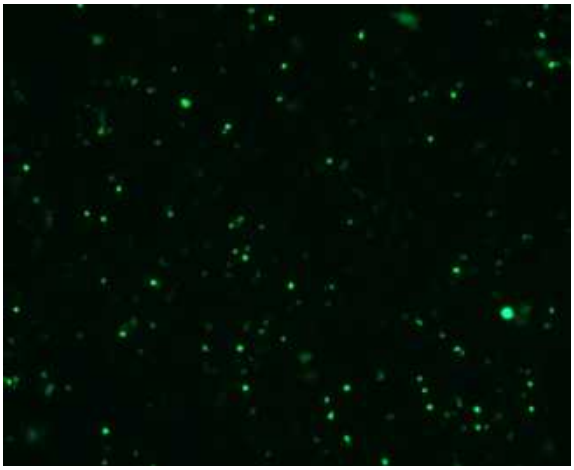
Kabeya Y, Mizushima N, Ueno T, Yamamoto A, Kirisako T, Noda T, Kominami E, Ohsumi Y and Yoshimori T. (2000) LC3, a mammalian homologue of yeast Apg8p, is localized in autophagosome membranes after processing. *EMBO Journal*, 19 (21): 5720-5728.

Appendix: Tables and/or figures:

Cells transfected with 1µg of DNA (pEGFP-C1) and 2 µl of Metafectene-Pro

HEP-3B





HCT-116