Transfection of Pancreatic Cell Lines for Diabetes Research using Metafectene Pro

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INTRODUCTION

Diabetes mellitus is a syndrome that results in an array of macro- and microvascular disorders that include coronary and cerebrovascular disease, renal disease, retinal disease, and neurologic disease. The International Diabetes Federation estimates that almost 250 million people world-wide have diabetes, and that the incidence is expected to increase in the coming years. Defects at the level of the insulin-producing pancreatic islets of Langerhans underlie all forms of diabetes. Thus, the study of isolated islets and pancreas-derived cell lines is of paramount importance in basic diabetes research. Whereas several such cell lines are available in the diabetes research community, virtually all lines display relatively poor DNA transfection efficiency in the range of 10-40% using currently available transfection reagents. Transfection efficiencies in the range of 70-100% are especially necessary for experiments involving RNA interference (using either double-stranded RNA or short hairpin RNAs) and transdifferentiation studies (where high efficiency gene delivery is necessary to observe population-based phenotypic changes in vitro). In our attempts to identify efficient, non-virus-based nucleic acid delivery vehicles, we compared transfection efficiencies in 3 commonly-used pancreatic cell lines (betaTC3, alphaTC1, and mPAC) and NIH3T3 cells using Metafectene Pro® transfection reagent and another reagent routinely used in our laboratory (Transfast®).

MATERIALS AND METHODS

Materials

All mouse pancreatic cell lines—beta cell line betaTC3 [1], alpha cell line alphaTC1 [2], and duct cell line mPAC [3]—were obtained from Dr. D. Hanahan (University of California, San Francisco). Metafectene Pro was obtained from Biontex Laboratories GmbH and Transfast was obtained from Promega Corp. The luciferase-encoding plasmid pCMV-Luc was obtained from Dr. M. German (University of California, San Francisco), and the enhanced green flourescent protein (EGFP)-encoding plasmid pEGFP-C1 was obtained from Clontech Laboratories, Inc.

Cell Culture and Transfection

betaTC3, alphaTC1, and mPAC cells were maintained in Dulbecco's modified Eagle medium (DMEM) supplemented with 15% horse serum, 2.5% fetal bovine serum, and 1% penicillin/streptomycin. The mouse fibroblast cell line NIH3T3 was maintained in DMEM supplemented with 10% calf serum and 1% penicillin/streptomycin. For transient transfections, $1x10^6$ betaTC3 and alphaTC1 cells, $5x10^5$ NIH3T3 cells, and

$1x10^5$ mPAC cells were seeded per well of 6-well plates 24 hours before transfection. A total 1 μg of plasmid was diluted with 100μl of PBS (Mix A). 3μl of Metafectene Pro was diluted with 100 μl of PBS and mixed gently by pipetting once (Mix B). Mix A and Mix B were combined and incubated 15 min at room temperature. During the incubation period, culture medium was removed from the cells and 2 ml of medium with serum (without antibiotics) was added to the cells. Transfection mixture was added to the cells and the 6-well plate was swirled gently. At 6 hours after transfection, the medium was changed to the normal growth condition medium. At 48 hours after transfection cells, were harvested using luciferase cell culture lysis reagent (Promega) and the luciferase assay was performed with the luciferase assay system according to manufacturer's instructions. Transfection with Transfast® reagent (Promega) was performed according the the manufacturer's protocol.

Live Cell Fluorescence Imaging

Live cell fluorescence images were obtained 48 hours after transfection using and Olympus IX81 Spinning Disk Confocal high speed 6D live cell microscope with Hammamatsu 9100-02 EMCCD camera and Orca ER deep cooled ccd cameras and Slidebook 4.2 software. For GFP imaging, fluorescence excitation occurred at 485nm.

RESULTS

Metafectene Pro reagent was used to transfect cells at a 3:1 reagent (μl) to DNA (μg) ratio; this ratio was demonstrated by the manufacturer to result in optimal transfection efficiencies in various cell lines. For transfections with Transfast® reagent, we used a 3:1 reagent to DNA ratio (which we have previously determined to be optimal for the cell lines used in this study). To ensure comparable results, we transfected a total of 1 μg of pCMV-Luc plasmid per well, regardless of reagent used, and normalized results to total protein. As shown in Fig. 1, use of Metafectene Pro® resulted a 6-, and 2-fold greater luciferase activity in βTC3 and mPAC cells, respectively, when compared to Transfast® reagent. An even more striking 17-fold greater luciferase activity in NIH3T3 cells was observed with Metafectene Pro compared to Transfast. AlphaTC1 cells showed no substantial increase in luciferase activity. Cellular toxicity for all cell types did not appear to be different between the two reagents, as assessed by cellular density (data not shown).

To determine the approximate efficiency of transfection with each of the two reagents, we performed fluroescence microscopy of cells transfeced with plasmid pEGFP-C1. As shown in Fig. 2, Metafectene Pro resulted in >70% of betaTC3 cells expressing EGFP, whereas fewer than 20% of betaTC3 cells expressed EGFP when transfected with Transfast. Increases in EGFP expression percentages with Metafectene Pro for mPAC and NIH3T3 cells were consistent with the increases

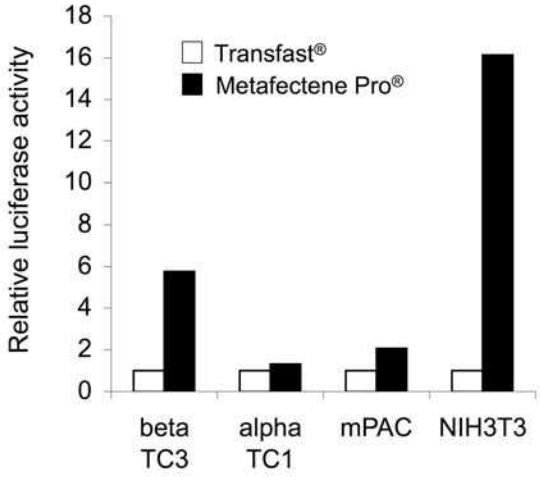

Fig. 1. Relative luciferase activities of betaTC3, alphaTC1, mPAC, and NIH3T3 cells transfected with plasmid pCMV-Luc. Data represent the average to two experiments, and are normalized to activities observed using Transfast® reagent.

observed with luciferase activities, whereas alphaTC1 cells showed no discernable increase in efficiency (see Fig. 2).

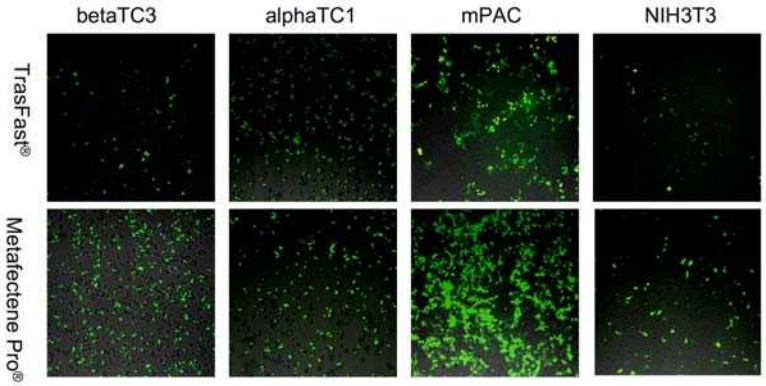

Fig. 2. Flourescence images of bTC3, alphaTC1, mPAC, and NIH3T3 cells transfected with plasmid pEGFP-C1. Live cell images were obtained 48 hours after transfection.

CONCLUSIONS

Metafectene Pro results in a substantially improved transfection efficiency in betaTC3, mPAC, and NIH3T3 cells compared to a commonly used alternative reagent. Although no substantial improvement in transfection efficiency was seen with alphaTC1 cells, Metafectene Pro® was not inferior.

REFERENCES

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