METAFECTENE PRO Mediated Gene Delivery to Lung Carcinoma Cell Line.

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As per World Health Organization reports, lung cancer is currently the most frequently diagnosed major cancer and the most common cause of cancer mortality in males worldwide (E. Brambilla et al. Eur Respir J 2001;18:1059–1068). Non small cell lung carcinoma (NSCLC) comprises the majority of lung cancer (over 75%) and when clinically extensive, it is typically characterized by inexorable disease progression despite treatment with chemotherapy and/or irradiation. A549 NSCLC is difficult cell line to transfect and offers resistance to most of the anticancer drugs (Brognard J. et al. Cancer Res. 2001 May 15;61(10):3986-3997). We delivered pEGFP-N_2 plasmid by using novel gene carrier Metafectene PRO in A549 NSCLC cell line and compared it with prototype cationic non-viral carrier PEI 25K. We measured GFP expression by FACS and confocal microscopy.

Materials and Methods

Materials

Metafectene PRO a polycationic liposomal transfection reagent was obtained from Biontex Laboratories GmbH (Munich, Germany). A549 (American Type Culture Collection) NSCLC cells maintained in RPMI 1640 supplemented with 10% FBS (Hyclone, South Logan, UT), 100 U/ml penicillin and cultured at 37º C in 5% CO_2 incubator. Plasmid pEGFP-N^2 with early promoter CMV and enhanced green fluorescence protein (EGFP) gene, was obtained from Clonetech.

Transfection protocol

A549 human lung adenocarcinoma cell line (2×10^5 cells/well) were seeded into each well of a 12 well plate (SPL Life Sciences) and allowed to attach overnight in RPMI 1640 growth medium containing 10 % fetal bovine serum, 100 U/ml penicillin. After

attaining 70-80% confluence cells were transfected with pEGFP-N_2 plasmid. Complex was prepared between Metafectene PRO and plasmid DNA (2µg) at ratios 2:1 and 4:1 by incubating for 30 min. PEI 25K transfection was performed at 10:1 N/P ratio. After complex formation, mixtures were diluted with serum free media to make final volume of 2 ml. The complexes were transferred to each well of 12 well plate and incubated with cells for 6 h, then the media was changed with fresh media containing serum and incubated at 37º C and 5% CO_2 for 48 h. Further transfection efficiency was evaluated by FACS caliber system and Enhanced green fluorescence was observed and recorded by confocal microscopy.

Results and Discussion

FACS analysis has shown 21% EGFP expression with 2:1 ratio which was three folds higher than PEI 25K while 32% EGFP expression was observed with 4:1 ratio which was 4.5 folds higher than PEI 25K. EGFP expression obtained by PEI 25K was 7% (Fig.1).

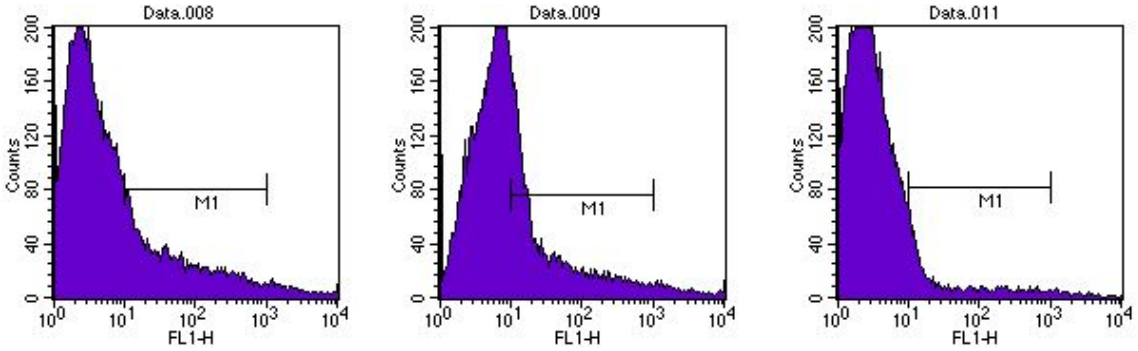

Figure 1. FACS experiment showed highest expression was obtained with Metafectene PRO at ratio 4:1.

Also similar results were obtained in confocal microscopic study, in which 4:1 and 2:1 ratios has shown very good EGFP expression much higher than PEI 25K as shown in Fig.2.

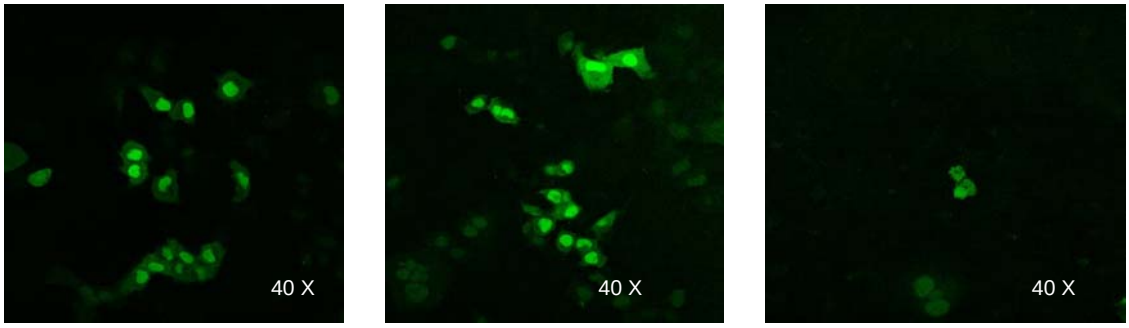

Figure 2. Confocal experiment showed highest expression was obtained with Metafectene PRO at ratio 4:1.

Conclusion

Metafectene PRO effectively transfect A549 cell line and may immerge as efficient non-viral vector in gene delivery.