Transfection of human neuroblastoma cells IMR32, Kelly, SH-SY5Y and La1-5S with Metafectene Pro

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Introduction:

In the Complement Biology Group in Cardiff University we have developed a new strategy for sensitisation of tumours to immunotherapy by treatment of cancer cells with peptides that suppress expression of complement regulators (Donev et al., Cancer Res, accepted; Yan J et al., Adv Exp Med Biol, 2008, in press). In order to investigate the potential therapeutic effect of our peptides we transfect cancer cells with plasmids that express the desired sequences. So far our research has been focused on neuroblastoma tumours. Neuroblastoma cells are relatively difficult to transfect. A number of reagents that give reasonably good transfection efficiency have been developed; however, these reagents are usually very toxic for this type of cells. Here we have set up experiments to investigate the transfection efficacy and cytotoxicity of the Metafectene Pro on several human neuroblastoma cell lines (IMR32, Kelly, SH-SY5Y and La1-5S).

Materials and methods:

Materials

Metafectene PRO, a polycationic liposomal transfection reagent, was obtained from Biontex
Laboratories GmbH (Munich, Germany). MTT Cell Viability Assay Kit was purchased from Cambridge Bioscience (Cambridge, United Kingdom). The penicillin, streptomycin and L-glutamine solutions were obtained from Invitrogen (Paisley, United Kingdom). The plasmid, pDsRed2-N1 vector (Clontech-Europe, France) encoding DsRed2 fluorescent protein was used for evaluating transfection efficiency.

Cells
Human neuroblastoma IMR32, SH-SY5Y, La1-5S and Kelly cell lines were obtained from the European Collection of Animal Cell Cultures (Salisbury, United Kingdom). They were maintained in RPMI 1640 with 10% heat-inactivated FCS, supplemented with L-glutamine (4 mM), penicillin (100 U/ml), and streptomycin (100 µg/ml) (Invitrogen).

Experimental procedures / transfection protocol:

Transfection protocol
For transfection, IMR32, Kelly, SH-SY5Y and La1-5S (1 x 105 cells/well) were seeded in 1 ml of growing medium in 48-well culture plates one day before transfection, and used at approximately 70-80% confluence. Cells were pre-washed with serum-containing RPMI 1640 medium and then covered with 0.9 ml of the same medium. Metafectene Pro was complexed with the pDsRed2-N1 plasmid at reagent:DNA ratios of 1µl:0.5 µg, 2 µl:0.5 µg or 2 µl:1 µg. Complexes were prepared by mixing Metafectene PRO with 100 µl of serum-free RPMI 1640 medium, followed by the addition of plasmid DNA. The mixture was incubated for 15 min at room temperature after the addition of the transfection reagent, and another 15 min after addition of DNA. Metafectene Pro complexes with DNA were added in a volume of 0.1 ml per well. Transfection efficacy was evaluated after 72 hours on a BD FACSCalibur (BD, Oxford, United Kingdom).

Cell viability assay
Cell viability (as a percentage of mocktreated control cells) was quantified by a MTT Cell Viability Assay Kit (Cambridge Bioscience) following the manufacturer instructions.

Results and discussion:

Metafectene Pro was complexed with the pDsRed2-N1 plasmid at reagent:DNA ratios of 1µl:0.5 µg, 2 µl:0.5 µg or 2 µl:1 µg. The highest percentage of cells expressing the DsRed2 fluorescent protein was obtained with 2 µl Metafectene Pro:1 µg DNA for all four studied cell lines (Fig. 1). However, we did not try further increase of the Metafectene Pro or the plasmid DNA, because our cytotoxic assay showed a substantial decrease in cellular viability after the ratio of 2 µl:0.5 µg (Fig. 2).

Conclusion / summary:

Metafectene Pro successfully transfected human neuroblastoma cell lines IMR32, Kelly, SH-SY5Y and La1-5S for which standard transfection reagents are very toxic. We determined that a ratio of 2 µl:0.5 µg between the reagent and DNA gave best results compromising between transfection efficiency and cytotoxicity.

References:

Yan J, Allendorf DJ, Li B, Yan R, Hansen R, Donev R. (2008). The role of membrane complement regulatory proteins in cancer immunotherapy. In Current Topics in Complement II, Lambris JD (Ed), Adv Exp Med Biol, Vol. 632, in press

Donev RM, Gray LC, Sivasankar B, Hughes TR, van den Berg CW, Morgan BP. (2008). Modulation of CD59 expression by REST-derived peptides in cancer immunotherapy for neuroblastoma. Cancer Res, accepted

Appendix: Tables and/or figures:

Figure 1. Optimisation of transfection efficiency in IMR32, Kelly, SH-SY5Y and La1-5S human neuroblastoma cell lines. The cells were transfected with the indicated amounts of Metafectene Pro and the pDsRed2-N1 plasmid. Results are expressed as percentage of cells expressing DsRed2 fluorescent protein as determined by flow cytometry. Data represent the mean ± S.D. obtained from triplicate wells, and are representative of two independent experiments.

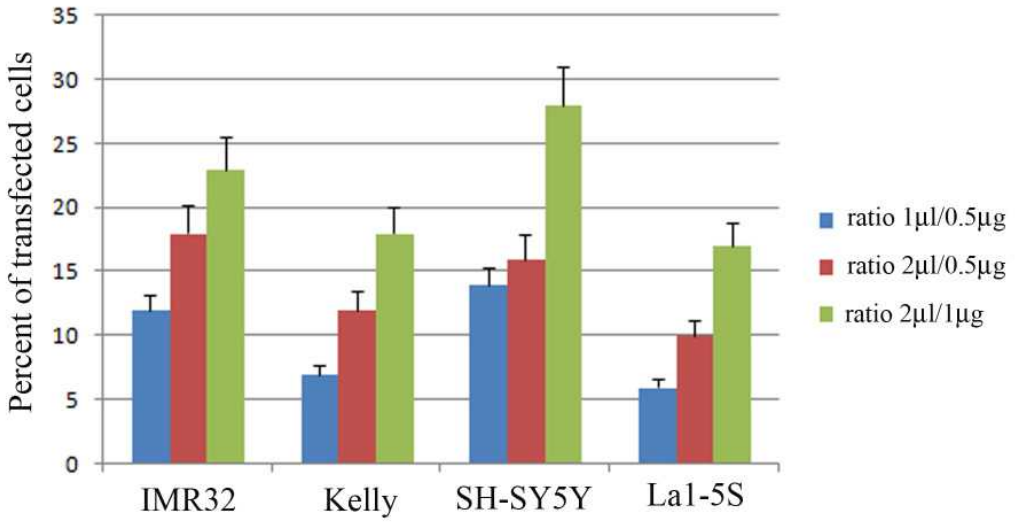

Figure 2. Effect of Metafectene Pro-mediated transfection with the pDsRed2-N1 plasmid on the viability of neuroblastoma cells was measured by the MTT Cell Viability Assay Kit. Results are expressed as percentage alive cells compared to a mock-transfected control. Data represent the mean ± S.D. obtained from triplicate wells, and are representative of two independent experiments.