

This article was downloaded by:

On: 26 January 2011

Access details: *Access Details: Free Access*

Publisher *Taylor & Francis*

Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Nucleosides, Nucleotides and Nucleic Acids

Publication details, including instructions for authors and subscription information:

<http://www.informaworld.com/smpp/title~content=t713597286>

Uptake and Intracellular Distribution of Oligonucleotides Vectorized by a PAMAM Dendrimer

Valérie Hélin^a; Marina Gottikh^{ab}; Zohar Mishal^c; Frédéric Subra^a; Claude Malvy^a; Marc Lavignon^a

^a UMR 1772, Institut Gustave Roussy, Villejuif, France ^b Belozersky Institute of Physico-Chemical Biology, Moscow State University, Moscow, Russia ^c CNRS IFC-01, Laboratoire de cytométrie, Villejuif, France

To cite this Article Hélin, Valérie , Gottikh, Marina , Mishal, Zohar , Subra, Frédéric , Malvy, Claude and Lavignon, Marc(1999) 'Uptake and Intracellular Distribution of Oligonucleotides Vectorized by a PAMAM Dendrimer', *Nucleosides, Nucleotides and Nucleic Acids*, 18: 6, 1721 — 1722

To link to this Article: DOI: 10.1080/07328319908044833

URL: <http://dx.doi.org/10.1080/07328319908044833>

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: <http://www.informaworld.com/terms-and-conditions-of-access.pdf>

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

UPTAKE AND INTRACELLULAR DISTRIBUTION OF OLIGONUCLEOTIDES VECTORIZED BY A PAMAM DENDRIMER

Valérie Hélin¹, Marina Gottikh^{1,2}, Zohar Mishal³, Frédéric Subra¹, Claude Malvy^{1,*} and
Marc Lavignon¹

¹UMR 1772, Institut Gustave Roussy, 39 Rue Camille Desmoulins 94800 Villejuif,
France.

²Belozersky Institute of Physico-Chemical Biology, Moscow State University, 117899
Moscow, Russia.

³CNRS IFC-01, Laboratoire de cytométrie, 94800 Villejuif, France.

ABSTRACT : We studied the uptake and intracellular distribution of an FITC labelled phosphodiester oligodeoxynucleotide (ODN) vectorized by a dendrimeric structure in cell culture.

INTRODUCTION

Factors limiting the use of antisense ODNs are inefficient cellular uptake and intracellular transport to RNA targets¹. A great number of chemical modifications have been proposed to increase ODN efficiency but they have raised problems related to ODN-sequence-specificity, stability of the ODN/RNA duplex and ODN ability to activate RNase H². Another means to enhance ODN activity is to vectorize them. We investigated, by flow cytometry, the ability of a polyamidoamine (PAMAM) dendrimer³ to enhance cellular uptake of both 3'- and 5'-FITC labelled 18-mer phosphodiester ODN on different cell lines. We also studied, by confocal microscopy, the intracellular distribution of the vectorized FITC-ODN.

RESULTS AND DISCUSSION

Flow cytometry showed that complexation of the FITC-ODN to a PAMAM dendrimer (SuperFectTM, Qiagen) strongly increased cellular fluorescence intensity which was greater on HeLa (human epitheloid carcinoma) and NIH 3T3 (murine fibroblast) cells compared

to CEM (nonadherent human lymphocyte) cells. Interestingly, a small population of cells (5 to 10 % of all the fluorescent cells) exhibited a strong intensity of fluorescence, at least 10 times higher than the intensity of the majority of fluorescent cells. After 30 minutes, fluorescent HeLa cells could already be detected and their percentage increased progressively during the first 4 h reaching a plateau of 80 to 90%. The cytotoxicity of the complex ODN-SuperFectTM was found to be insignificant on HeLa cells and approximately 30% on NIH 3T3. Confocal microscopy showed, with both adherent cell lines, an heterogeneity of the intracellular distribution of fluorescence whatever the time of incubation with the vectorized FITC-ODN (from 1 h to 24 h). Some cells exhibited perinuclear fluorescence and others, strong nuclear fluorescence. Controls carried out on cells with FITC alone and the analysis of FITC-ODN extracted from cells by PAGE showed that the fluorescence observed inside cells corresponded to the FITC-ODN intact.

In conclusion, we found that the dendrimeric structure permits the FITC-ODN to enter efficiently into cells. Moreover, in order to optimize ODN activity, it appears relevant to study in depth of the subpopulation of cells, observed by flow cytometry, presenting a high fluorescence intensity and the one, detected by confocal microscopy, showing a nuclear localization of FITC-ODN⁴.

ACKNOWLEDGMENTS : This study was supported by grants from the Association pour la Recherche contre le cancer (ARC, n° 6317) and from the Russian Foundation for Basic Research (n° 98-04-22055). V. Hélin is supported by the Institut de Formation Supérieure Biomédicale (IFSBM) and the Centre National de la Recherche Scientifique (CNRS).

REFERENCES

1. Bennett C.F. *Biochem. Pharmacol.*, **1998**, *55*, 9-19.
2. Stein C.A., Tonkinson J.L. and Yakubov L. *Pharmacol. Ther.* , **1991**, *52*, 365-384.
3. Bielinska A., Kulowska-Latallo J.F., Johnson J., Tomalia D.A. and Baker J.R. *Nucleic Acids Res.*, **1996**, *24*, 2176-2182.
4. Moulds C., Lewis J.G., Froehler B.C., Grant D., Huang T., Milligan J.F., Matteuci M.D. and Wagner R.W. *Biochemistry*, **1995**, *34*, 5044-5053.