Polymer-mediated gene transfection efficiency is positively correlated with polyplex-induced autophagy in fibroblasts

X. Zhong, D. Panus, C. Wang

Department of Biomedical Engineering, University of Minnesota, Minneapolis, MN 55455, USA
Presenting author: zhong072@umn.edu

ABSTRACT SUMMARY

The aim of this work is to gain deeper understanding of the mechanism of cationic polyplex-induced cytotoxicity and its impact on polyplex-mediated gene transfection. Here we report that autophagy in murine fibroblasts could be induced by polyethylenimine-mediated gene transfection. Enhancing cell autophagy with rapamycin led to increase in transfection efficiency, while blocking autophagy with inhibitor 3-methyladenine decreased transgene expression. Importantly, the effect of autophagy on transfection efficiency was independent from apoptosis and necrosis, did not involve reactive oxygen species (ROS), and was potentially affected by subcellular trafficking pathways. These findings suggest the possibility of enhancing gene transfection efficiency by exploiting autophagic cytotoxicity.

INTRODUCTION

Three major pathways to cytotoxicity are apoptosis, necrosis and autophagy [1]. Unlike apoptosis, autophagy (or “self-eating”) is often considered a cell survival mechanism through removing damaged proteins and subcellular organelles, so as to cope with stressful conditions such as starvation and chemical or biological assaults [2]. Autophagy induction has also been observed in certain cell types exposed to various cationic molecules alone [3]. Because many cationic polymers are also gene transfection reagents, it is important to know whether polymer/DNA complexes could induce autophagy, and how induced autophagy might affect gene transfection. Furthermore, because the cellular stress responses of autophagy and apoptosis are often intertwined [4], it is desirable to assess polyplex-induced autophagy independently from polyplex-induced apoptosis.

In this study, we investigated autophagy induction by PEI/DNA polyplexes in murine fibroblasts and attempted to decouple polyplex-induced autophagy from apoptosis and necrosis and evaluated the influence of autophagy on polyplex-mediated transgene expression using small molecules known to inhibit or induce autophagy.

EXPERIMENTAL METHODS

Polyplex, transfection, & modulation of autophagy. Polyplex of branched PEI (25 kDa) and GFP plasmid was prepared at N:P ratio of 8. NIH 3T3 mouse fibroblasts were transfected for 4 h in serum-free media followed by 20 h of culture in complete media. Transfection efficiency was measured by quantification of GFP expression by flow cytometry. To inhibit autophagy, 10 mM of 3-methyladenine (3-MA) was incubated with the cells during the 4-h transfection. To promote autophagy, 100 nM of rapamycin was added 2 h before and 4 h after transfection with polyplex.

Detection and quantification of autophagy. Twenty-four hours after transfection, autophagy in fibroblasts was characterized using (1) transmission electron microscopy (TEM), (2) immunostaining with LC3 antibody followed by fluorescence microscopy, and (3) Western blot for LC3 (I and II).

Apoptosis and necrosis and ROS. Transfected cells were stained for Annexin V and propidium iodide and sorted by flow cytometry. ROS level in cells 4 and 24 h post-transfection was quantified using 2′,7′-dichlorofluorescin diacetate (DCFH-DA) oxidation and microplate reader.

Cellular uptake and subcellular trafficking. Cells were transfected with polyplexes consisting Cy5-labeled Luciferase plasmid and cellular uptake, colocalization with autophagosome (stained for LC3), endosomal entrapment, and nuclear uptake at were quantified using confocal fluorescence microscopy and image analysis as previously described [5].

RESULTS AND DISCUSSION

TEM images of transfected cells revealed the presence of autophagosomes for their characteristic double-membrane structure (Fig. 1). Transfected cells also showed cytoplasmic clustering of LC3, a marker for autophagosomes (Fig. 2). The number of cells with autophagosomes and the level of LC3 II were both significantly higher in transfected cells compared to untreated cells (Fig. 2,3). Furthermore, 3-MA and rapamycin reduced or enhanced autophagy (Fig. 2,3) without affecting apoptosis/necrosis (~5% cells).

Fig. 1 TEM images of transfected cells showing autophagosomes with double-membrane structure.
Transfection efficiency of fibroblasts by PEI/DNA complexes was around 20-30% GFP+ cells (Fig. 4). Inhibiting autophagy with 3-MA resulted in a dramatic reduction of transfection efficiency from ~26% to ~6%, and even more in mean fluorescence intensity (MFI). In contrast, inducing autophagy with rapamycin led to a modest increase in GFP+ cells and a two-fold increase in MFI (Fig. 5). Notably, this effect on transfection efficiency was attributed only to autophagy modulation without interference from apoptosis/necrosis, nor did it change ROS production. Preliminary subcellular trafficking data suggest the involvement of altered intracellular processing of polyplexes by autophagic cells.

CONCLUSION
Polyplex-mediated transfection induced autophagy in murine fibroblasts. The efficiency of transfection was positively correlated with autophagy induction independent from apoptosis/necrosis, and was possibly regulated by altered subcellular trafficking pathways in autophagic cells.

REFERENCES
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