Drug delivery of miR-539-5p for treatment of Glioblastoma using dendrimer nano-particles

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Abstract

Objectives: In this study we used Poly(amido-amine) (PAMAM-OH) generation four as a nano-carrier system to deliver miR-539-5p efficiently to cancer cells.
Methods: PAMAM-OH-G4 was internally quaternized with different degrees that was calculated using NMR-spectrometry, then assembled with miR-539-5p with different N/P ratios under fixed conditions. Then, characterized using Zetasizer. The encapsulation efficiency and releasing of free miRNA at conditions resembling human cells were determined using gel electrophoresis and RiboGreen assay, respectively. U87 human brain glioma cells were transfected with the miR-539-5p dendriplexes to determine the transfection efficiency and therapeutic effects in in vitro using flow cytometry and MTT assay, respectively.
Results: The average size of miR-539-dendriplex was 12.95±5 nm, and zeta potential of +29.6±4.88 mV. The encapsulation efficiency was almost 100% with N/P ratios 16. Treatment with QPAMAM-OH dendrimer alone and assembled with miR-NC showed no cytotoxic activity on U87 cell line.
Conclusion: QPAMAM-OH is a safe and effective tool used to deliver miRNA to target cells. Our data showed cellular internalization of QPAMAM-OH at rate almost 80% on U87. Additionally, it showed no cytotoxic activity in U87 cell line.

Introduction

Glioblastoma is the most frequently diagnosed malignant human glioma, and current median survival is less than two years despite maximal treatment. MicroRNAs have been demonstrated to be deregulated in different cancers. Functional analysis of miR-539 in glioma revealed that miR-539 expression was significantly decreased. This study infers that miR-539 overexpression inhibits the proliferation and invasion of glioma cells by directly suppressing the disheveled-axin (DIX) domain containing 1 (DIXDC1), which is emerged as a novel oncogene in GBM that is highly upregulated in glioma cancer tissues. However, miRNAs have difficulty penetrating through cell membranes and are vulnerable to degradation in the blood stream. Poly(amido-amine) (PAMAM) was a good carrier due to due to its high solubilization, efficient cellular internalization, sustained release and low toxicity.
Polyamidoamine (PAMAM) hydroxy-terminated generation 4-6 dendrimers have shown high uptake and uniform distribution into the entire brain tumor. These neutral dendrimers have demonstrated a unique pharmacokinetic characteristic in a glioblastoma model after systematic administration as they selectively accumulate and retain in tumor tissue.
In this study, we prepared nanocomplexes with miR-539 using QPAMAM-OH G4 and study the effect of these complexes on glioblastoma cell lines.

Methodology

➢ Preparation and Characterization of miR-539-5p dentriplexes

QPAMAM-OH dendrimer (Dendritech,Michigan, USA) with 43% degree of quaternization was prepared and the degree of quaternization was determined by NMR (JOEL, 400 MHz). The has-miR-539-5p (RiboBio,China) solution (5 µmol/L) was added to QPAMAM-OH-G4 solution at N/P ratio of 16 and 33. The mixture then incubated for 24 h with vigorous shaking at 4ºC. The encapsulation efficiency was determined using agarose gel 2% stained with ethidium bromide. The samples were added to the gel. Then subjected to 120 V for 30 min in 1X TAE buffer. The particle size and zeta potential was determined by TEM microscopy and Zetasizer (Malvern Instrument Ltd, Malvern UK), respectively.

➢ Cellular Internalization

U87 cells 2 x 10^5 were seeded in 6-well plate with complete media for 24h, then transfected with FITC-QPAMAM-OH-miR-539-5p dendriplexes for 5h. Part of the cells then harvested and the FITC fluorescence was detected by flow cytometry (BD FACS CANTO II). The other part washed then fixed with 50%, 75%, and 100% methanol, the Nuclei were dyed with DAPI (Thermo, USA) for 10 min. The cells then visualized using EVOS FL Auto from life technologies microscope.

➢ Transfection and Proliferation Assay

U87cells 5 x 10^3 were seeded in 96-well plate with complete media for 24h, then starved with 1% FBS advanced DMEM for 24h. Cells then transfected with miR-539-5p dendriplexes for 48h. Then MTT.

Result

➢ Preparation and Characterization of miR-539-5p dentriplexes

The average size of miR-539-dendriplex with N/P 16 and 33 was 12.95±5 and 20.3 ±6.5 nm, respectively. And zeta potential was +29.6±4.88 and+25±8.15 mV, respectively. **Table.1**
Retardation of miRNA indicates that the encapsulation efficiency was approximately 100%. **Figure. 1**

N/P Ratio	Zeta potential (mV)	Particle size (nm)
16	+29.6 ± 4.88	12.95 ± 5
33	+25 ± 8.15	20.3 ± 6.5

Tab.1

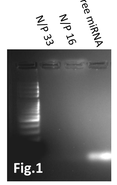


Fig.1

➢ Transfection and Proliferation Assay

Constant pars indicated that there is no cytotoxic activity of QPAMAM-OH dendrimer both assembled and non-assembled on U87. **Figure.4**

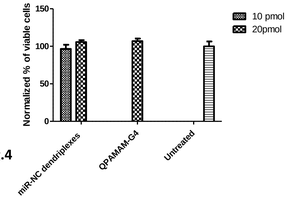
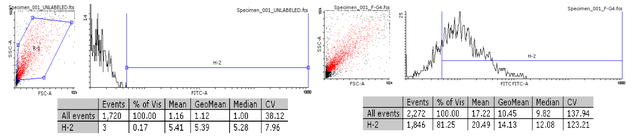


Fig.4

➢ Cellular Internalization

Positively labeled cells showed 81% ratio measured by flow cytometry. **Figure. 2.**
Positive transfection is depicted using FITC-dendriplexes (green) while cells' nuclei were labeled with DAPI (blue). **Figure. 3**



	Events	% of Vis	Mean	GeoMean	Median	CV
All events	1,720	100.00	1.16	1.12	1.00	38.12
H-2	3	0.17	5.41	5.39	5.28	7.96

	Events	% of Vis	Mean	GeoMean	Median	CV
All events	2,272	100.00	17.22	10.45	9.82	137.94
H-2	1,846	81.25	20.49	14.13	12.08	123.21

Fig.2

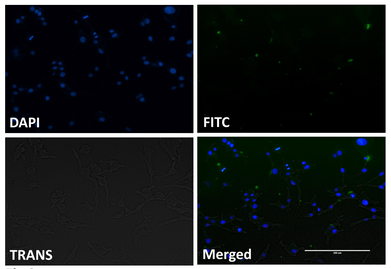


Fig.3

Conclusion

- Average size of miR-539-dendriplex with N/P 16 and 33 was 12.95±5 and 20.3 ±6.5 nm, respectively.
- Cellular uptake of miR-539-dendriplex was almost 81%.
- FITC-dendriplexes were detected representing positive uptake.
- QPAMAM-OH has no cytotoxic effect on cancer cells.

References

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