#176 Antitumor effects of cationic dendritic molecules and their complexes with microRNA in glioblastoma stem-like cells

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Background: Manipulations with miR34a and onco-miR21 demonstrated their effectiveness as antitumor intervention in glioblastoma (GBM), so stable and efficient nanocarriers should be found. One of the promising approach is using of dendrimers – highly symmetrical hyperbranched polymers. The aim of our study was evaluation of effects of treatment by complexes of dendrimers with miRs (dendriplexes) on human glioblastoma stem-like cells (GSCs).

Materials and methods:

Cells:

- Glioblastoma cell culture (U87 as a suspension culture);
- Glioblastoma stem-like cells cultures (BTSC233, JHH520, NCH644, GBM1);
- iPS cells.

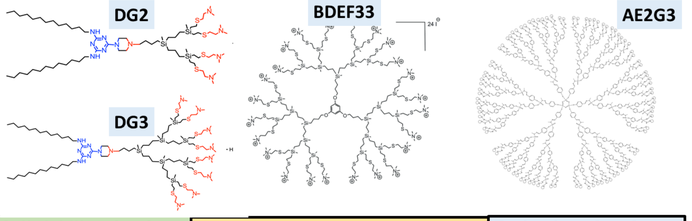
Cationic dendritic molecules (DMs) – free or in complexes with miRs according to charge ratio :

- 2nd (DG2) and 3rd (DG3) generation carbosilane dendrons;
- 3rd generation fully symmetrical carbosilane dendrimer (BDEF33);
- 3rd generation fully symmetrical phosphorus dendrimer (AE2G3).

microRNAs (miRs):

- miR34;
- Synthetic inhibitor of miR21 (amiR21).

25 nM, 50 nM, 100 nM, 150 nM



Cell viability after free DMs treatment in comparison with temozolomide (TMZ):

- MTT assay (72 h);
- Annexin V/PI assay (72 h).

Evaluation of dendriplexes internalization:

- FACS (3 h)

Cell viability after dendriplexes treatment:

- Annexin V/PI assay (72 h).

Evaluation of surface markers expression – PD-L1, TIM3, CD47, CD133:

- FACS (3 h)

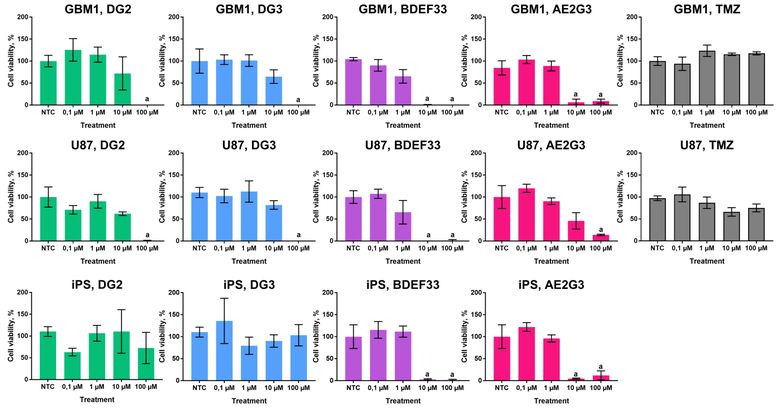


Fig.1. Evaluation of cell viability after treatment by free dendrimers (72 h). "a" marks significant difference with non-treated control (NTC) (p<0,05).

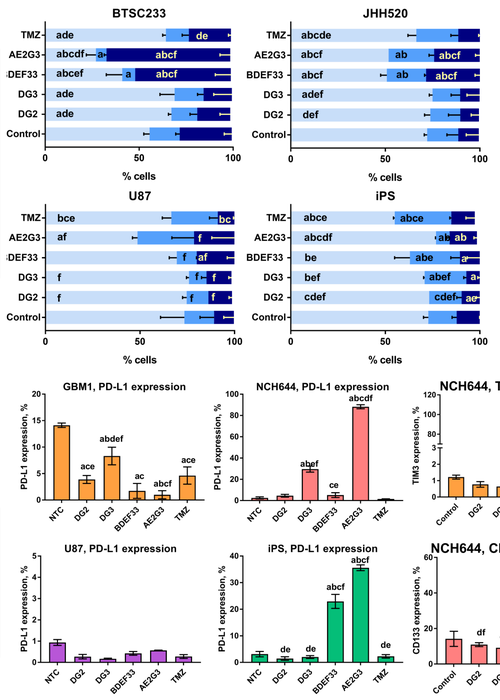


Fig.2. Evaluation of apoptosis after treatment by free dendrimers and dendriplexes (72 h). "a" marks significant difference with non-treated control (NTC); "b" – in comparison with DG2; "c" – in comparison with DG3; "d" – in comparison with BDEF33; "e" – in comparison with AE2G3; "f" – in comparison with TMZ (p<0,05).

- Late apoptotic/Dead
- Early apoptotic
- Live cells

Fig.3. Evaluation of expression of surface markers on cells after treatment by free dendrimers (72 h). "a" marks significant difference with non-treated control (NTC); "b" – in comparison with DG2; "c" – in comparison with DG3; "d" – in comparison with BDEF33; "e" – in comparison with AE2G3; "f" – in comparison with TMZ (p<0,05)

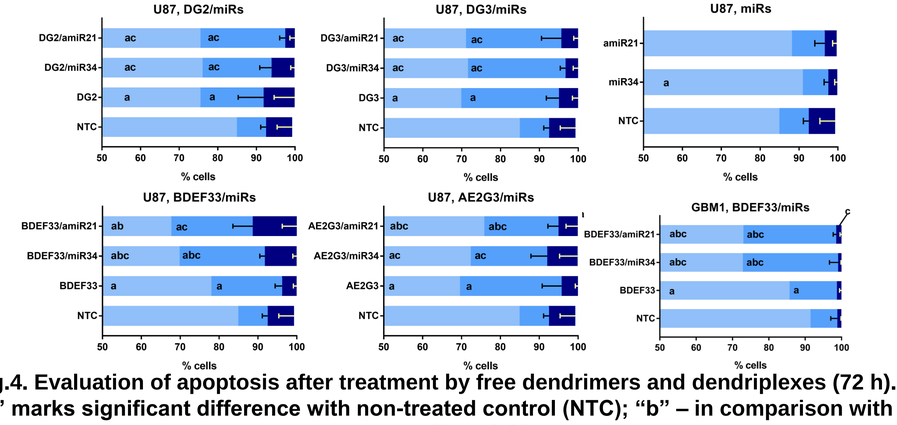


Fig.4. Evaluation of apoptosis after treatment by free dendrimers and dendriplexes (72 h). "a" marks significant difference with non-treated control (NTC); "b" – in comparison with free carrier; "c" – in comparison with free miR (p<0,05).

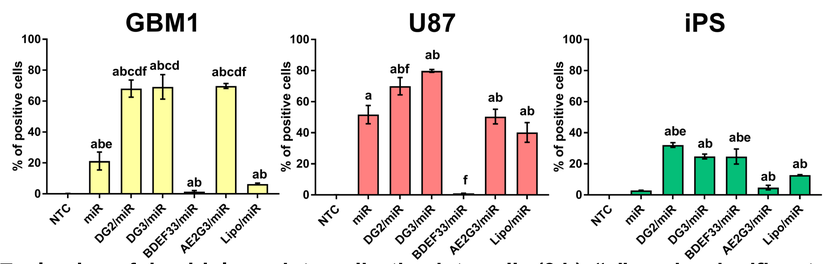


Fig.5. Evaluation of dendriplexes internalization into cells (3 h). "a" marks significant difference with non-treated control (NTC); "b" – in comparison with free carrier; "c" – in comparison with free miR; "d" – in comparison with Lipofectamin3000/miR complex; "e" – in comparison with internalization into U87; "f" – in comparison with internalization into iPS (p<0,05)

Results:

- DMs demonstrated their own anti-tumor activity, which was shown to be higher than for TMZ in GSCs cultures.
- Treatment by free DMs led to significant changes of PD-L1, TIM3, CD133 expression.
- Molecules under study could be used as efficient miRs carrier into tumor cells.
- Complexes containing amiR21 decreased GBM1 cells viability and reduced PD-L1 expression

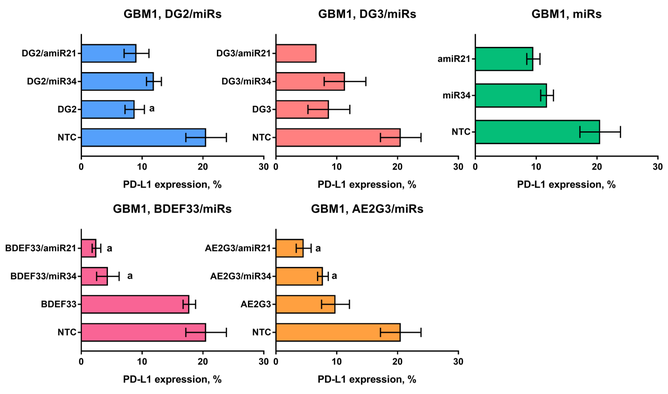


Fig.6. Evaluation of expression of surface markers on cells after treatment by dendriplexes (72 h). "a" marks significant difference with non-treated control (NTC); "b" – in comparison with free carrier; "c" – in comparison with free miR (p<0,05).

Conclusions

Cationic DMs can be potentially used as effective components of antitumor therapy in GBM either alone or as the carriers of therapeuthic nucleic acids. However their effects on expression of surface molecules interacting with tumor microenvironment deserve further studies.

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