Development of Nanoconjugate with Different Monoclonal Antibodies to Inhibit Molecular Targets Important for Tumor Angiogenesis

M. Fujita[*], N. M. Khazenzon[*], B.-S. Lee[*], E. Holler[**,***], K. L. Black[*,***] and J. Y. Ljubimova[*,***]

[*] Maxine Dunitz Neurosurgical Institute, Cedars-Sinai Medical Center, 8631 West Third Street, Suite 800E, Los Angeles, CA 90048, USA, ljubimovaj@cshs.org
[**]Universität Regensburg, Regensburg, Germany, eggeho@gmx.de
[***]Arrogene Inc., Tarzana, USA

ABSTRACT

Two antisense oligonucleotides blocking α and β chain mRNAs of an invasive tumor marker, laminin-8, were delivered simultaneously to brain tumor cells via receptor-mediated endocytosis using Polycefin [3]. Polycefin variants bearing different antibodies to transferrin receptor and/or human anti-nuclear autoantibody 2C5 show significantly different accumulation in the tumor. In the xenogeneic model used, the variant with the combination of mouse anti-TfR and human anti-TfR provides the most effective drug delivery through mouse endothelial system and into implanted human brain tumor cells. Another efficient variant was Polycefin containing the combination of mouse anti-TfR and human anti-nucleosome antibody 2C5. Presence of two or more different antibodies on Polycefin at the same time may be important for future drug delivery and therapeutic efficacy of the tumor treatment.

Keywords: Polycefin, anti-transferrin receptor antibodies, human anti-nuclear autoantibody 2C5, Morpholinos antisense oligonucleotides, laminin-8, tumor angiogenesis.

INTRODUCTION

Cell-specific drug targeting is the major goal of nanotechnology-based platforms. This is particularly relevant in anticancer therapy that is limited by side effects that arise from toxicity to normal cells. Because of the inherent limitations associated with passive enhanced permeability and retention effect-based targeting, the next generation of drug delivery systems is being engineered with ligands targeted against cell surface antigens [1]. Polymers able to deliver inhibitory agents to tumor cells increasingly gain importance because they are less immunogenic than viral vectors and more useful for repetitive treatments important for angiogenesis inhibition [2]. A new prototype drug delivery system, the nanoconjugate Polycefin, was synthesized for targeted delivery of antisense oligonucleotides and antibodies into tumors. Imaging analysis showed that the delivery system passed through the blood-brain and blood-tumor barriers [3, 4]. The delivery of antisense oligonucleotides to α4 and β1 chains of a vascular basement membrane protein, laminin-8 suppressed its synthesis *in vivo*. Treatment of glioma-bearing rats significantly diminished tumor vascularity ($p<0.001$) and significantly increased animal survival ($p<0.004$) [5].

MATERIAL AND METHODS

Polycefin consists of modules active in endosomal uptake, endosomal membrane disruption, oligonucleotide release in the cytoplasm, protection from enzymatic degradation, and fluorescent dye Alexa 680 for imaging. Two anti-tumor antibodies were tested, anti-nuclear autoantibody 2C5 specific for tumor cells [6], and anti-transferrin receptor (TfR) antibody. All these components were covalently conjugated to highly purified poly(malic acid) (M_w 50000, M_w/M_n 1.3) from *Physarum polycephalum*. The presence of two different antibodies on Polycefin was confirmed by HPLC and ELISA. Human glioma cell line U87MG was inoculated intracranially into nude mice. Polycefin variants were injected intravenously. Xenogen IVIS 200 Imager was used to detect and quantitate Polycefin accumulation *in vivo* (Fig. 1-4).

RESULTS

Several Polycefin variants were tested (Fig. 1): a. Polycefin with anti-mouse TfR antibody, b. Polycefin with anti-human TfR antibody, c. Polycefin with both antibodies to TfR, and d. Polycefin with human anti-nuclear autoantibody 2C5 alone, and e. Polycefin with autoantibody 2C5 together with anti-mouse TfR. Drug accumulation was higher in tumor and adjacent area than in normal non-tumor brain (cortex and cerebellum, Fig. 2). Drug accumulation with only anti-mouse antibody was significantly lower in tumor and adjacent area (Fig. 2 A) than with only anti-human TfR antibody (Fig. 2 B, C). The highest tumor accumulation was achieved when the drug contained the combination of 1. anti-mouse and anti-human TfR antibodies, or 2. anti-mouse TfR and human anti-nuclear autoantibody 2C5 (Figs. 3, 4). These differences were significant ($p<0.001$).

Fig. 1 Polycefin variants by targeting monoclonal antibodies

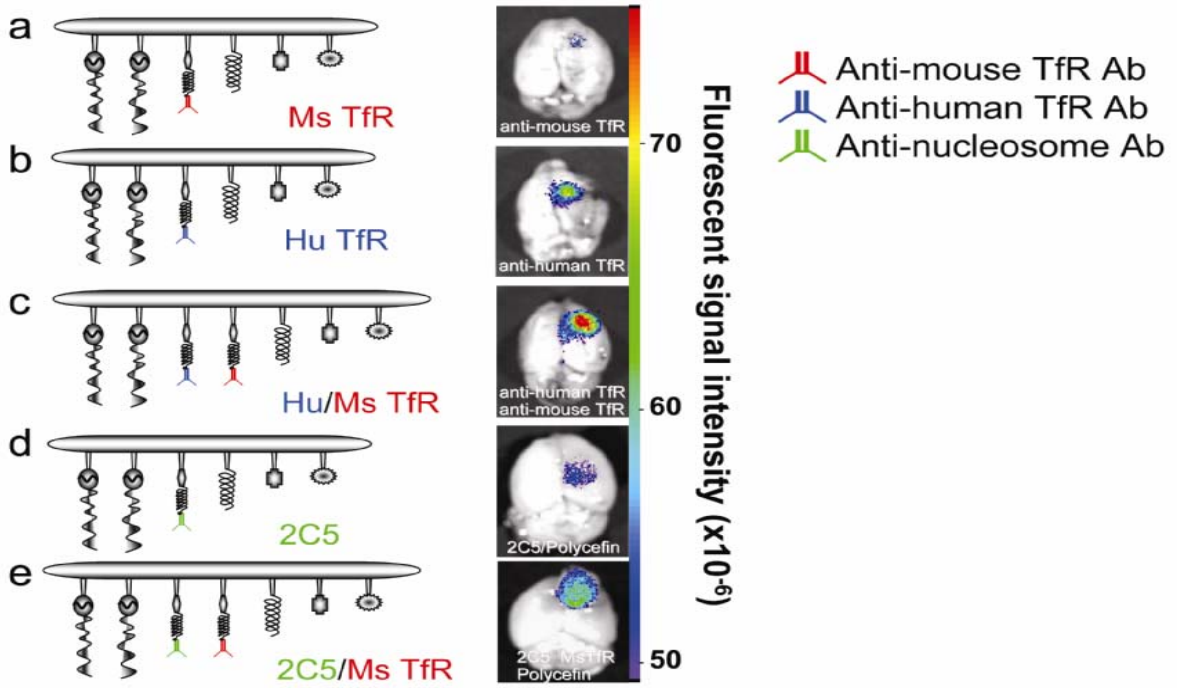


Fig. 2 Imaging study

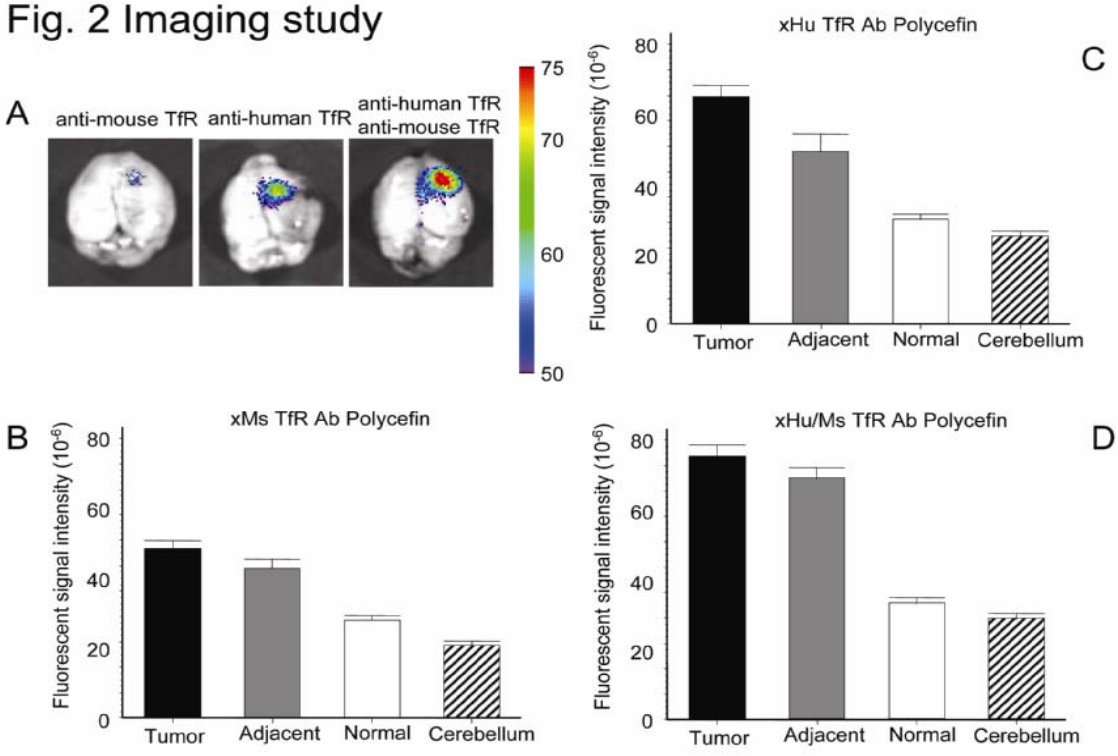
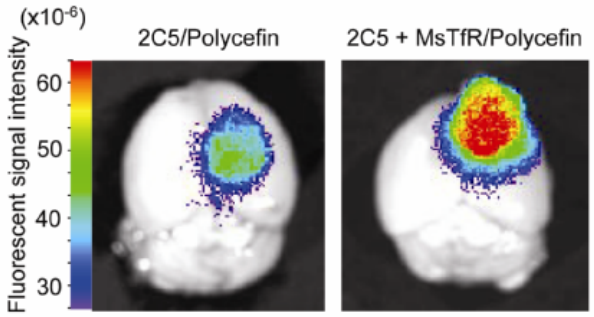


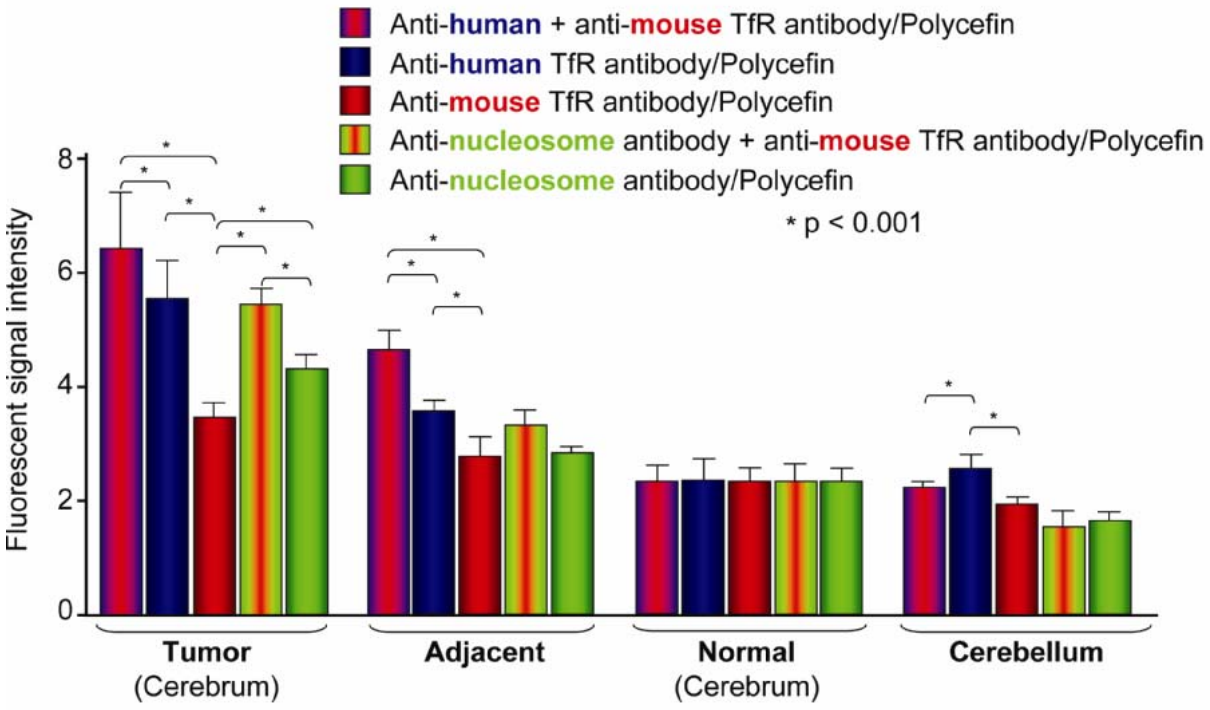
Fig. 3 Polycefin accumulation using antibodies against MsTfR and 2C5



CONCLUSION

More than a single kind of antibody can be conjugated to the Polycefin drug carrier platform. The examples of mounted mAbs are side by side functionally active, one in the permeation of mouse blood-tumor-barrier (BTB) and the other in targeting the implanted human brain tumor. Both human anti-transferrin antibody and antibody 2C5 recognizing a general human tumor cell surface antigen express tumor targeting activity. Presence of two or more different antibodies on Polycefin at the same time may be important for future drug delivery and therapeutic efficacy of the tumor treatment.

Fig. 4 Polycefin accumulation in tumor, adjacent tissue, normal cerebrum and cerebellum



REFERENCES

[1] S.P. Leary et al., Neurosurg., 6, 1009-1026, 2006.
[2] R. Satchi-Fainaro, et al., Nat. Med., 10, 255-261, 2004.
[3] B.-S. Lee, et al., Bioconjugate Chem., 17, 317-326, 2006.
[4] J.Y. Ljubimova, et al., Chemical Biological Interactions, 2007 in press.
[5] M. Fujita, et al., Angiogenesis, 9, 183-191, 2006.
[6] V.P. Torchilin, et al., Cancer Ther., 1, 179-190, 2003.