Nature Reviews Drug Discovery | AOP, published online 19 November 2010; doi:10.1038/nrd3332

DRUG DELIVERY

Nanobioconjugate shrinks brain tumours

Gliomas are among the most lethal types of cancer, and treatment options are very limited. Now, Ding and colleagues present a multifunctional nanobioconjugate that is capable of carrying antisense oligonucleotides (AONs) across the blood–brain barrier (BBB) and the blood–brain tumour barrier (BTB), targeting its payload to the tumour for receptor-mediated cellular uptake, endosomal escape and release into the cytoplasm. Using this construct, the authors demonstrated dramatic inhibition of intracranial tumour growth in xenograft mouse models of human gliomas.

The principal limitations in the design of nanobioconjugates are biodegradability, toxicity, immunogenicity and the ability to deliver drugs to the cytoplasm. These challenges were addressed by creating a universal drug delivery platform based on poly(β-L-malic acid), or PMLA, a non-toxic, non-immunogenic polymer derived from the slime mold *Physarum polycephalum*.

To facilitate drug delivery across the BTB, antibodies targeting the transferrin receptor were covalently attached to the polymer (for the xenograft experiment, this involved the tandem coupling of anti-mouse and anti-human transferrin receptor antibodies). The construct was shown to cross the BTB by transcytosis and to directly enter tumour cells, where it underwent receptor-mediated endocytosis. A pH-dependent endosomal escape unit, based on a bacterial lysogenic trileucine peptide, was added to the construct to facilitate exit into the cytoplasm and to prevent subsequent degradation in lysosomes. A tracking dye and moieties to optimize solubility were also added. Finally, the payload of two different AONs, directed against the α4 and β1 subunits of laminin-411 — which is overexpressed in the tumour neovasculature and plays a role in angiogenesis, but is difficult to inhibit with conventional methods — were attached with a disulphide linker that is cleaved by glutathione in the cytoplasm.

In vitro experiments with the nanobioconjugate resulted in efficient cellular uptake and endosomal release, and knock down of laminin-411 production. Double-labelling of the backbone and the AONs showed co-localization in endosomes, which decreased over a 3 hour incubation period, indicating endosomal escape and cytoplasmic release of the AONs. Microscopic analysis of brain slices from treated mice showed accumulation of the construct in the brain tumour, and a knock down of laminin-411 expression. After eight rounds of intravenous injection every 3 days, tumours of treated mice were 90% smaller than those of control mice, and their blood vessels resembled those in healthy brain tissue. Compared with a construct with a constitutively active endosomal escape unit, pH-restricted membranolysis was shown to increase bioavailability and reduce cytotoxicity, resulting in significantly improved efficacy.

These experiments demonstrate that PMLA provides an exciting platform for specific tumour targeting and precise intracellular inhibitor delivery, and validate laminin-411 as a target for the treatment of glioma. The authors point out that PMLA-based delivery systems meet the criteria of safety and efficacy, and that variants of this system might also offer promising strategies for other neurodegenerative conditions such as multiple sclerosis or Alzheimer's disease.

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ORIGINAL RESEARCH PAPERS Ding, H. *et al.* Inhibition of brain tumor growth by intravenous poly(β-L-malic acid) nanobioconjugate with pH-dependent drug release. *Proc. Natl Acad. Sci. USA* **107**, 18143–18148 (2010)