Local delivery of recombinant proteins from encapsulated cells reduces glioma growth

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INTRODUCTION

Drug delivery to the brain is a major challenge for any brain disorder including brain neoplasms. The delivery of substances from blood to brain is tightly regulated by the blood-brain barrier (BBB) which is composed of endothelium with tight junctions resting on a basal lamina, perivascular cells and astrocytic end feet. In the intact brain the BBB prevents free diffusion of molecules into the brain parenchyma and passage is largely regulated by specific transporter molecules. In malignant brain tumours, extensive neovascularization leads to a partially compromised BBB and vascular leakage. However, elevated interstitial fluid pressure within the tumors frequently restricts drug delivery to these areas. Also, the invasive front of the tumor border is very difficult to reach by systemically administered compounds. Thus, drug-based brain tumor treatment requires methods that overcome these limitations. Current strategies include the development of drugs that pass the BBB or co-administration of drugs that modulate BBB permeability (e.g. bradykinin). Therapeutic factors can be infused into the surgical cavity either through direct injections or through convection-enhanced delivery (CED) which involves slow infusion rates in the brain parenchyma via intracranial catheters. More recent delivery strategies circumventing the BBB involve the implantation of engineered neural and mesenchymal stem cells making use of the homing capacity of these cells.

The implantation of micro-encapsulated producer cells in or near the resected tumor cavity represents an alternative approach with the added value of ensuring continuous and controlled drug release at the tumor site. The encapsulation device allows bi-directional diffusion of nutrients, oxygen and waste, while at the same time providing an immuno-isolating environment that prevents the encapsulated cells from being destroyed by immunocompetent host cells. In the brain, this approach leads to a long-term *de novo* delivery of therapeutic proteins, thus circumventing the need for high systemic drug concentrations and repeated surgical interventions.

Micro-encapsulation of cells is designed for the delivery of cell-made products including endogenous proteins or peptides, or engineered chimeric proteins. Here we propose to apply a soluble receptor protein that interferes with tumor neo-angiogenesis in a rodent model of malignant glioma.

Glioma angiogenesis

Malignant gliomas are among the most well vascularised tumours in man and extensive pathological neovascularisation is a common finding in malignant glioma. Vascular proliferation constitutes one of the criteria for increased malignancy grade in the present WHO classification of brain tumours. Increased microvascular density (MVD) is also reported to be a negative prognostic

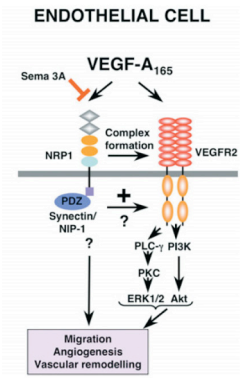
factor in malignant glioma. The neoplastic vessels found in malignant glioma are pathologic with a chaotic organisation, variable lumen diameter, increased permeability and variable tumour blood flow (Jain *et al*, 2007). The pathologic architecture of the tumour vascular bed gives rise to one of the most problematic clinical features of malignant glioma, the vasogenic brain oedema, where corticosteroids remain the most widely accepted treatment. Angiogenesis, the formation of new blood vessels from pre-existing ones, is the most prominent mechanism by which new vessels are formed in a clinically detectable growing glioma (Carmeliet & Jain, 2000).

Vascular endothelial growth factor (VEGF) is generally considered to be the most important positive regulator of angiogenesis today. It was originally discovered as a potent vascular permeability factor but subsequently also found to be an important endothelial cell mitogen. The VEGF family as known today consists of five members found in man (VEGF-A, -B,-C,-D and PlGF). VEGF-A (also often reffered to as VEGF) is extensively expressed in high grade gliomas and is believed to be the most important angiogenesis factor in malignant glioma.

In malignant glioma several experimental studies indicate that angiogenesis inhibition in general, and inhibition of the VEGF signalling pathway in particular, may be a successful treatment of malignant glioma. The combined VEGF-R/EGF-R TKi vandetanib, has been shown to inhibit experimental glioma growth and potentiate the effects of chemotherapy and radiotherapy (Sandstrom *et al*, 2008). In the clinical setting, bevacizumab has recently been shown to inhibit progress of relapsing glioma together with the topoisomerase-1 inhibitor irinotecan in a small phase II study (Vredenburgh *et al*, 2007). Bevacizumab has also been combined with a standard temozolomide based radiochemotherapy setting and phase II data are encouraging (Lai *et al*, 2008).

Neuropilins

The neuropilins (Nrp-1 and Nrp-2) are co-receptors for class 3 semaphorins and are involved in axonal guidance during development where they act as negative regulators. NRP-1 and NRP-2 both bind the semaphorin class 3 ligands and form complexes with plexin-A. Neuropilins have also been shown to bind certain VEGF isoforms and form complexes with VEGF-Rs enhancing VEGF-R signalling (Pellet-Many *et al*, 2008). Initially NRP-1 was believed to be an isoform specific receptor for $VEGF_{165}$ (Soker *et al*, 1998) but recent work indicates that also $VEGF_{121}$ binds NRP-1 although unable to form complexes with VEGF-Rs. VEGF-B and -E have been shown to bind NRP-1 whereas the lymphangiogenic VEGF-C and D can bind NRP-1 as well as NRP-2.



The neuropilin receptors NRP-1 and -2 are widely expressed in different human cancers and cell-lines and the expression of neuropilins is induced by several growth factors including VEGF and EGF. Hypoxia has been reported to induce the expression of neuropilins in some models but the results are somewhat contradictory and some studies indicate a negative regulation of NRP-1 expression upon hypoxia. In malignant glioma NRP-1 is expressed by both tumour cells and endothelial cells.

Figure 1: Schematic representation of VEGF signaling in endothelial cells via VEGFR2 and neuropilin-1 (Nrp1). Soluble Nrp1 bodies corresponding to the extracellular domain of Nrp1 have been shown to block VEGFR2 signaling by ligand scavenging.

MATERIAL AND METHODS

Constructs: Lentiviral vector constructs expressing soluble Nrp1 bodies (sNrp1) have been obtained through a collaboration with J. Verhaagen (Netherlands Institute for Neuroscience, Amsterdam) and R. Giger (Univeristy of Rochester, US).

Encapsulation of cells: Cells were genetically engineered by lentiviral vector transduction to express soluble neuropilin-1. In some cases cells were also expressing a fluorescent marker protein: green fluorescent protein (GFP) or Discosoma red fluorescent protein (DsRed). This allows the direct visualization of the capsules *in vitro* and *in vivo* and to follow cell survival over time. The producer cells (C_2C_{12} or BHK) were harvested and mixed into a 2% sodium alginate (ultrapure, low viscosity, high guluronic acid content; PRONOVA (TM) UP LVG) saline solution (0.9% NaCl, 10mM MOPS, pH 7.4) to give a concentration of 50×10^6 cells/ml alginate solution. Beads were generated with an electrostatic bead generator. Sharpened nozzles with diameter of 0.17 mm were used (Nisco Engineering AG, Switzerland). The gelling bath was composed of 0.1M $CaCl_2$ in 0.9% NaCl with 10mM MOPS, pH 7.4. After encapsulation the cell line released sNrp1 from the micro-capsules, verified by Western blot analysis (data not shown).

Animal model: U87 glioma cells stably expressing firefly luciferase were injected into the brain of nude mice. At the same time sNP1-expressing cell capsules were implanted into the injection site. Luciferase gene allowed the in vivo monitoring of tumour growth using a bioluminescent imaging system (IVIS Lumina, Calipers). Tumors were followed for 3 weeks, animals were sacrificed and tumor volume was assessed ex vivo by MRI.

Immunohistochemistry was performed to visualize tumor blood vessels and tumor cell distribution. Western blot analysis was done according to standard procedures.

RESULTS

We have implanted alginate-based microcapsules (Fig.2) containing recombinant producer cells to interfere with tumor blood vessel formation and/or tumor cell proliferation. We will present data from two mouse models showing effective reduction in tumor volume in the presence of anti-angiogenic proteins released from cell microcapsules

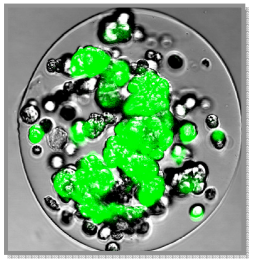


Figure 2: BHK cells encapsulated in alginate beads.

Mice were injected with Control U87 cells or soluble Nrp1 (sNrp1) expressing U87 cells were injected into the brain of nude mice. The cells stably expressed firefly luciferase (luc2) which allowed visualization of the growing tumor in the living mice, using a bioluminescent imaging system (IVIS Lumina, Calipers). sNrp1 lead to a drastic reduction of tumor growth. The same experiment was performed using sNrp1 producer cells encapsulated in alginate beads and co-implanted with the U87 tumor cells. Cell capsules were sufficient to dramatically reduce tumor growth in this model (data not shown).

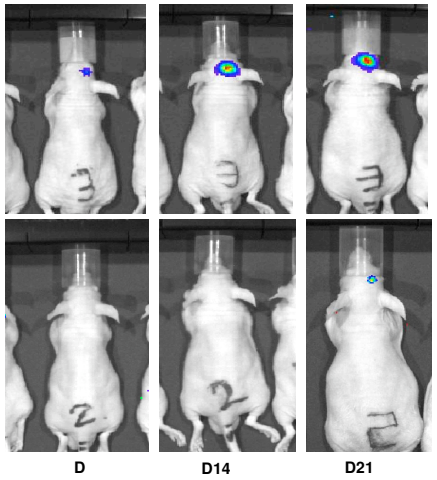


Figure 3: Tumor growth visualized by bioluminescent imaging. Upper row: control U87-luc2 glioma. Lower row: U87-luc2 tumors expressing sNrp1. D: day3, day 14 day21 after implantation.

CONCLUSIONS

Our results provide evidence that the application of cell capsules concomitantly adminitrered during brain surgery may be a powerful new approach to deliver therapeutic agents against malignant gliomas. Our data also supports the promising approach of anti-angiogenic therapy against malignant gliomas. The infiltrative and highly angiogenic glioblastoma xenograft model developed in our laboratory will be invaluable to investigate the potency of multi-factorial bioreactors delivering a combination of therapeutic substances (*Sakariassen et al. 2006 ; Niclou et al. 2008*).

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