Development and Characterization of EGFR-targeted Iron-oxide Nanoparticles for Improved Magnetic Resonance Imaging of Brain Tumors.

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Magnetic resonance imaging (MRI) is a prevailing medical imaging technique for solid tumors, because of its non-invasive nature and high soft-tissue contrast. In the clinics, contrast agents, such as iron oxides or gadolinium chelates, are administered to enhance the MRI signal by increasing the proton relaxation times of tissue water [1]. These agents passively accumulate at the tumor site due to the altered vessel architecture commonly observed in cancerous tissue; a phenomenon referred to as enhanced permeability and retention (EPR) effect.

Glioblastoma multiforme (GBM), also named fourth grade astrocytoma, is the most abundant and severe type of primary brain neoplasm with a mean patient survival of 14 months. At the present, surgical resection in combination with adjuvant radio- and chemotherapy (temozolomide) is the standard GBM therapy applied in the clinics [2]. Patient outcome is expected to improve with efficacious removal of the tumor mass; therefore precise delineation of tumor outlines is of key importance to the brain surgeon.

The epidermal growth factor receptor (EGFR) is overexpressed by gene amplification in half of GBM patients; herein, the expression of a constitutively active mutant receptor subtype (EGFRvIII) is observed in 40% of the cases and has shown to correlate with poor prognosis (*Figure 1*) [3]. EGFR belongs to the ErbB family of receptor tyrosine kinases, whose members are crucially involved in signaling pathways that regulate cell proliferation, differentiation, survival and angiogenesis. In particular, the highly invasive cells at the tumor boundaries display increased levels of EGF receptors on their surface [4].

In this study we developed an EGFR-specific superparamagnetic iron oxide nanoparticle, which is anticipated to improve the visualization of tumor outlines by MR imaging. The small size of the nanoprobe may improve tissue penetration and allow for the detection of infiltrating cancer cells in the surrounding brain. Furthermore, receptor-mediated internalization of the contrast agent into the cells is expected to improve accumulation and retention at the tumor site, resulting in prolonged high T2-contrast in brain lesions.

Novel anti-EGFR camelid antibody fragments were generated to target the nanoparticulate contrast agent to the tumor site [5]. Here, we demonstrate the tumor cell targeting ability of these 15 kDa antibody fragments linked to commercially available iron oxide nanoparticles in a glioblastoma cell line model (*Figure 2*). Furthermore, we carried out a thorough characterization of the conjugated nanoparticles, investigating its stability, toxicity, magnetic properties, blood circulation times and *in vitro* and *in vivo* targeting ability.
This nanoparticulate contrast agent could later on be exploited as multi-modal imaging agent, combining the benefits of MR imaging and fluorescence labeling for intra-operative visualization of tumor cells.

References:

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[5] Zhang J, Tanha J, Hirama T, Khieu NH, To R, Tong-Sevinc H, Stone E, Brisson JR, MacKenzie CR. J Mol Biol. 2004 Jan 2;335(1):49-56.

Figures:

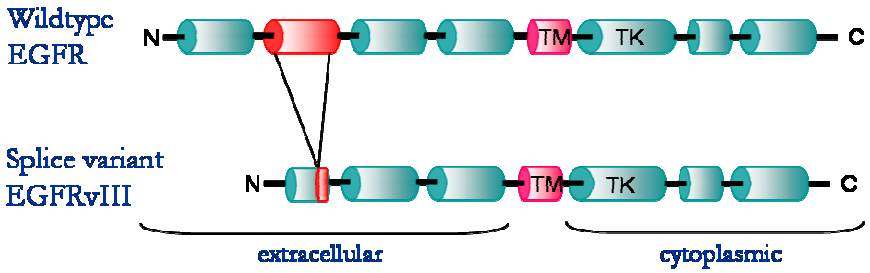

Figure 1. *Schematic representation of the extra- and intracellular domains of the epidermal growth factor receptor (EGFR) and the mutant receptor (EGFRvIII).*

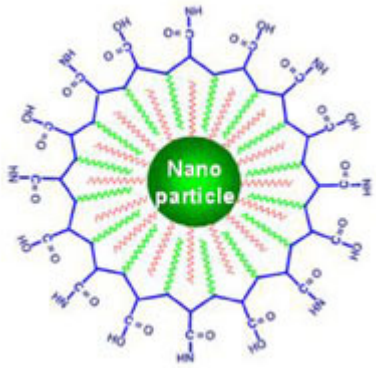

Figure 2. *Schematic representation of the surface modifications on superparamagnetic iron oxide nanoparticle obtained from Ocean NanoTech, Springdale, AR, USA.*