Evaluation and optimization of the concept of an antibody directed enzyme-prodrug therapy using noninvasive imaging technologies


(1) MI for exp Medicine, Göttingen, Germany
(2) University Medicine Göttingen, Germany
(3) University Göttingen, Germany
(4) MPI for exp Medicine, Göttingen, Germany

jnowako1@gwdg.de

Introduction: Relatively low selectivity to tumor versus normal cells challenges virtually all cancer chemotherapies. Site-specific activation of prodrugs in tumors is one strategy to achieve high efficacy and specificity of treatment, decreasing toxicity in normal tissues. Here, we present the design and validation of an Antibody Directed Enzyme-Prodrug Therapy (ADEPT) in which an antibody against Eag1 is used to carry the drug-activating enzyme, β-galactosidase (β-gal) to the tumor tissue. Eag1 (ether-α-go-go1) voltage-gated potassium channel has been chosen as a tumor-specific target since this plasma-membrane protein is easily accessible to extracellular interventions and Eag1 is aberrantly expressed (>75%) in tumors from diverse origin but basically not detected in healthy tissue outside the central nervous system.

Methods: Two monoclonal anti-Eag1 antibodies, mAb62 and mAb56, a humanized 56 antibody, as well as a single chain fragment of the mAb62, scFv62, were tested for their feasibility to deliver the drug-activating enzyme β-gal to the tumor. Near infrared fluorescence (NIRF) imaging was used to study the biodistribution and binding characteristics of the anti-Eag1 antibodies in vivo in a subcutaneous Eag1-expressing MDA-MB-435S tumor model in nude mice. Fluorescence intensity, lifetime and location of Cy5.5 labeled antibodies in vivo was measured with the time-domain NIRF imager, Optix MX2 (ART, Advanced Research Technologies; Canada), at certain time points. Fluorescence lifetime (the average time during the molecule stays in its excited state) was used to discriminate between non-specific and probe-derived signals. Distribution of the fluorescent probe in tumor sections ex vivo was measured with the time-domain NIRF imager, Optix MX2 (ART, Advanced Research Technologies; Canada), as well as by NIRF microscopy (Axiovert 200M, Carl Zeiss, Germany). For the tumor-specific activation of the prodrug, the mAb62 was conjugated to β-gal, resulting in 62-gal. The β-gal activity of the 62-gal conjugate and its ability to bind to the Eag1 epitope were tested in vitro on Eag1-expressing MDA-MB-435S cells and control AsPC-1 cells using a colorimetric CPRG (chlorophenolred-β-D-galactopyranoside) assay. The β-gal activity in mice was analyzed by NIRF imaging using the fluorescent activatable probe, DDAOG (9H-(1,3-dichloro-9,9-dimethylacridin-2-one-7-yl) β-d-galactopyranoside) by NIRF imaging.

Results: All tested antibodies targeting Eag1 bound specifically to MDA-MB-435S tumors with maximal intensity peaks at 24-48 h after application; fluorescence was still detectable for at least 1 week in vivo. We confirmed specific binding of the antibodies to the tumors by ex vivo NIRF imaging of tumors isolated from mice injected with fluorescently labeled antibodies 24 h prior to section, as well as by NIRF microscopy of tumor slices. Moreover, we show that monoclonal antibodies against Eag1, but not the scFv62 fragment, resulted in strong fluorescence signals in the area over liver, detectable for at least 4 days in vivo. Similar fluorescence signals over liver were also observed in tumor-bearing mice injected with Cy5.5-labeled control IgGκ2B, confirming that the liver signals did not result from Eag1-mediated binding of those antibodies to cells within liver. Furthermore, we show that the 62-gal conjugate specifically binds in vitro to Eag1-expressing MDA-MB-435S, but not to control Eag1-non expressing AsPC-1 cells and that 62-gal possess high β-gal activity, when bound to MDA-MB-435S cells. Moreover, 24 h after application of the 62-gal to the tumor bearing mice we detected β-gal activity in vivo over the tumor area.

Conclusions: Here, we successfully applied NIRF imaging to evaluate anti-Eag1 antibodies as tools for a novel concept of targeted cancer therapy. Since in vivo 62-gal 1) specifically binds to Eag1-expressing tumors and 2) shows measurable β-gal activity at the tumor site, this conjugate can further be applied in the ADEPT for specific activation of cytotoxic prodrugs at the tumor site.