Assessment of HIF transcriptional activity in a mouse tumor model using GPI anchored avidin– a novel protein reporter for *in vivo* imaging

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Introduction: With the emergence of multimodal imaging approaches genetic reporters, which can be flexibly combined with multiple imaging methods, are highly attractive. Here we present the feasibility of using glycosylphosphatidylinositol anchored avidin (av-GPI) (1) as a novel reporter for multimodal *in vivo* imaging. Expressed on the extracelluar side of cell membranes, av-GPI can be targeted with biotinylated imaging probes. In this study, we employed av-GPI to read out on the activity of hypoxia inducible factors (HIFs) in tumors. Induced by tumor hypoxia to mediate the adaptation of cells to low oxygen tensions these transcription factors play an important role in cancer progression. Typically, the expression of HIF in human cancer patients is associated with more aggressive tumor phenotypes and poor patient prognosis. Imaging HIF activity in live tumors hence provides an important tool to study the mechanisms leading to its activation in cancer.

Methods: Mouse C51 cells were stably transfected with pH3SVG, a reporter construct driving the expression of avidin-GPI from a minimal SV40 promoter and 3 hypoxia response element (HRE), bound by HIF, from the human transferrin gene (2). To monitor HIF activity *in vivo*, pH3SVG transfected C51 cells were subcutanouesly implanted into Balb/C nude mice. 10 days after tumor inoculation, mice received an i.v. injection of alexa-594-biocytin and were imaged using fluorescence reflectance imaging.

Results: Fluorescence stainings of av-GPI expressing cells demonstrated that this protein is specifically expressed on the extracellular side of cell membranes. Moreover, upon pharmacological activation of HIF, we observed a shift in fluorescence indicative of an increased expression of av-GPI in fluorescent activated cell sorting (FACS) experiments involving pH3SVG transfected cells. *In vivo* fluorescence imaging showed a specific uptake of a biotinylated dye (alexa-594-biocytin) in the tumor from 60 minutes after contrast injection, whilst there was no accumulation of an unbiotinylated control probe (alexa-594-cadaverine). On ex vivo tissue sections alexa-594 biocytin was found to co-localize with zones positive for pimonidazole, a commonly used hypoxia marker (3) but also showed staining in regions devoid of pimonidazole uptake. In additional experiments, biotinylated 67Ga-DOTA was shown to specifically label avidin expressing cells in vitro.

Conclusions: Overall, we demonstrate the utility of av-GPI as a reporter for *in vivo* imaging of HIF transcriptional activity in an optical, fluorescence reflectance approach. In vitro binding studies with 67Ga-DOTA showed a high specificity of the probe in targeting av-GPI in cells, which implies that this reporter can indeed be combined with different imaging modalities. Its application in SPECT is currently being tested.

References:

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