In vivo imaging of radioarsenic labelled anti-PS antibody with small animal PET

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Introduction: Phosphatidylserine (PS) is a phospholipid, which under normal conditions resides almost exclusively in the inner leaflet of the plasma membrane. PS asymmetry is maintained by an ATP-dependent aminophospholipid translocase that is responsible for inward movement of aminophospholipids. Loss of PS asymmetry results from the outward movement of PS in the plasma membrane and is caused either by the inhibition of the translocase or activation of scramblase, a Ca^{2+}-dependent enzyme that transports lipids bidirectionally and is observed under different pathologic and physiologic conditions, including programmed cell death (apoptosis), cell aging and cell migration [1]. Vascular PS exposure is observed in solid tumors as a result of exposure to stress conditions in the tumor microenvironment. Injury and activation of tumor endothelium are known to be caused by tumor-associated cytokines, leukocytes, metabolites, and by hypoxia followed by reoxygenation (cf. Fig. 1). Therefore antibodies against PS should be ideal for tumor targeting, especially for the targeting of tumor vasculature endothelium [2]. The ch3G4 anti-PS antibody was labelled with the PET isotope ^{74}As ($T_{1/2}$=17.77 d, 29% β^+) as described in [3] and was applied to small animal PET imaging at various time points.

Experimental: The antibody was SATA-modified and labelled as described in [3]. *In vivo* experiments were carried out in R3327-AT1 Dunning prostate tumor bearing male Copenhagen rats. To obtain tumors, small tumor pieces, excited of a donor animal, were implanted subcutaneously in the right thigh of the rat. Tumor-bearing rats were injected intravenously via the lateral tail vein with 100 µg of [^{74}As]SATA-ch3G4 in 4 ml PBS (100 µCi per rat). PET measurements were performed after 24 h and 72 h p.i. under general anaesthesia for 4 hours.

Results and Discussion: SATA-ch3G4 was labelled with ^{74}As with a radiochemical yield > 95%, verified via HPLC. The specific activity achieved was 5.56 GBq/µmol. The antibody mainly targets apoptotic cells in tumor endothelium, as shown via immunochemistry (cf. Fig. 1). This matches with the results, as the tumor has a capsule-like structure with a highly vasculatured outer ring and a less vasculatured inner core, where there is less detectable AB than in the outer ring of apoptotic tissue on the edge of becoming necrotic, which was verified via necropsy and necrosis-staining of tumor tissue. The longer-living ^{74}As provides the possibility to monitor pharmacokinetics of this AB for the first time over a longer period. Biodistributions and phosphor storage imaging *in vivo* and of tumor slices were also performed and are described in [3].

The low available activity of 100 µCi per rat was not enough to achieve sufficient data for a whole body scan or 3D-reconstruction as time of measurement is limited by the anaesthesia of the rat.

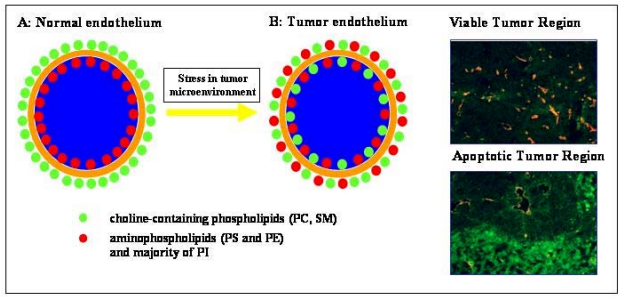

Fig.1: Exposure of the major phospholipids of the plasma membrane on normal (A) and loss of asymmetry on tumor (B) endothelium. The immunostaining shows the localization of 3G4 in mice bearing MDA-MB-435 human breast tumors for viable and apoptotic tumor tissue. The green color indicates the 3G4 Anti-PS antibody and the red color Anti-CD31 marked endothelium.

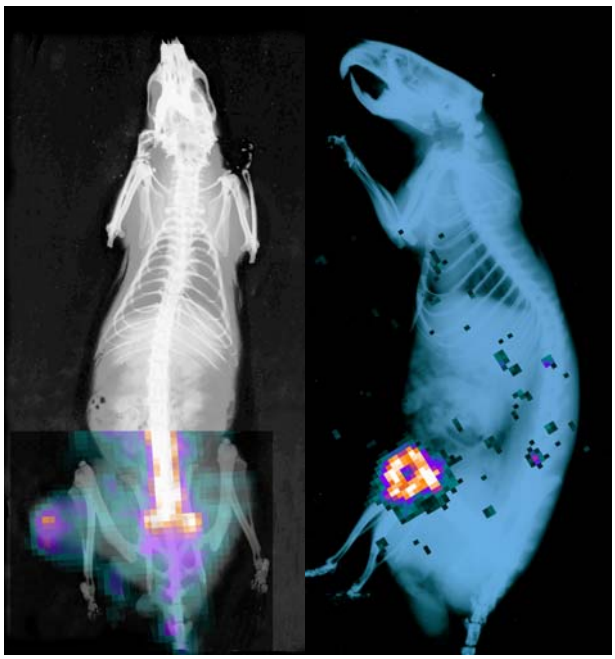

Fig. 2: [^{74}As]SATA-ch3G4 small animal PET, 24 hours (ventral projection) and 72 hours (lateral projection), overlayed with x-ray images.

Conclusion: For the first time, a radioarsenic labelled antibody could be studied *in vivo*. The PET-images show an impressive tumor enrichment. ^{74}As might not be the ideal isotope for PET, because of its low positron emission rate of 29%, but the long half-life of 17.8 days nevertheless makes it a useful tool for the observation of long-term pharmacokinetics.

References:

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