Activated leukocyte cell adhesion molecule (ALCAM) as a potential MRI biomarker for detection of brain micrometastases

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Introduction

The incidence of brain metastasis from primary cancers such as breast, lung and melanoma is increasing. Clinically, diagnosis and treatment of these tumours in the early stages is challenging since the intact blood-brain barrier (BBB) limits access for both imaging contrast agents and systemic therapies. The aims of this study are: (1) to evaluate activated leukocyte cell adhesion molecule (ALCAM) as a potential target for brain micrometastasis detection; and (2) to develop a new MRI contrast agent based on microparticles of iron oxide (MPIO) and anti-ALCAM antibodies.

Methods

Antibodies against ALCAM were conjugated to MPIO (ALCAM-MPIO) and antibody loading assesed using flow cytometry. ALCAM-MPIO binding to ALCAM was verfied on mouse endothelial cells treated with TNF, and also under flow conditions in capillaries coated with mouse recombinant ALCAM protein. Mice (SCID, n=24) were injected intracardially with $1x10^5$ human brain tropic breast cancer (MDA-MB-231-Br; n=12) or melanoma (H1-DL2; n=12) cells. On imaging day, mice were injected intravenously with ALCAM-MPIO and scanned using a T_2*-weighted multi-gradient echo sequence. Clinically relevant pre- and post-gadolinium T_1-weighted images were also acquired. Mice were perfusion-fixed and brains taken for immunohistochemistry.

Results/Discussion

Successful coupling of the ALCAM-MPIO conjugate was confirmed (22,000 Ab/MPIO). ALCAM-MPIO showed significantly ($p<0.001$) higher binding to both pre-activated endothelial cells and capillaries compared to MPIO conjugated to an equivalent, but non-specific IgG antibody. *In vivo* MRI of both tumour models showed focal hypointensities on T_2*-weighted images, indicating presence of ALCAM-MPIO. No gadolinium enhancement was detected at these sites. Co-localisation of MRI hypointense signals with micrometastases and bound ALCAM-MPIO was verified histologically.

Conclusion

The results of this study indicate that ALCAM may be a potential target for early detection of brain micrometastases. We have demonstrated ALCAM-specific binding of a new molecular MRI contrast agent (ALCAM-MPIO) both *in vitro* and *in vivo*, in two different models of brain metastasis. Together these data suggest that ALCAM-MPIO in combination with MRI may provide a new approach for the early detection of brain metastases, prior to blood-brain barrier breakdown, and could substantially improve diganosis for patients at risk of secondary progression to the brain.