

MECHANISTIC PATHWAYS UNDERLYING ALTERED NEUROVASCULAR COUPLING IN BRAIN METASTASIS

Cerebrovascular Regulation

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Abstract

Objectives: It is recognised that astrocytes form a physical bridge between neurons and the cerebrovasculature. Astrocytic end-feet help maintain endothelial tight junctions to support the blood–brain barrier (BBB) and release vasoactive molecules that regulate vascular tone. In secondary cancer (metastasis) to the brain, astrocytes are displaced from the vasculature and become activated, and we have shown that neurovascular coupling is compromised in rat model of brain metastasis (Serres et al., abstract submitted). The aim of this study, therefore, was to characterise the effects of astrocyte activation on astrocyte-vascular structure, vascularity and production of vasoactive molecules, using in vivo magnetic resonance imaging (MRI) and immunofluorescent microscopy.

Methods: Two cohorts of BD-IX rats were injected intracortically in one node of the whisker barrel cortex pathway (the barrel field somatosensory cortex) with either (i) a lentivirus expressing ciliary neurotrophic factor (Lv-CNTF) known to switch astrocytic phenotype to an activated state, or (ii) a metastatic N-ethyl-N-nitrosourea-induced mammary adenocarcinoma cell line (ENU1546). Lv-CNTF injected animals were studied 6 weeks after intracortical injection, and ENU1546 injected animals were studied 1 week after injection. All animals underwent T1- and T2-weighted MRI to follow macroscopic structural changes, and post-gadolinium T1-weighted MRI to assess BBB integrity. Immunofluorescent microscopy was performed post-mortem to identify activated astrocytes (GFAP),

neurons (NeuN), blood vessels (CD31), cyclooxygenase-1 and 2 (COX-1/2), inducible isoform of nitric oxide synthase (iNOS), glutathione (GSH), cytochrome p450 a precursor of 20-hydroxyeicosatetraenoic acid (20-HETE), alpha-smooth muscle actin (α -SMA) and proteoglycans of the basement membrane (β -dystroglycan).

Results: In both cohorts, persistent activation of astrocytes, revealed by strong upregulation of GFAP, was observed in the injected cortex, which was not associated with BBB disruption as assessed by MRI. Disruption of the dystroglycan-laminin interaction was observed in the area of astrocyte activation causing dissociation of astrocytes from blood vessels, as shown by a lower immuno-colocalisation of blood vessel, astrocyte and β -dystroglycan, for both Lv-CNTF and ENU1546 injected animals (Fig1). Enlargement of blood vessels was also observed in both models. Together with structural changes in the astrocyte-vessel complex, upregulation of iNOS, COX-1, COX-2 and GSH were observed in activated astrocytes. Whilst upregulation of cytochrome p450 was observed in α -SMA-positive arterioles found in the area of astrocyte activation.

Conclusion: Our findings suggest that astrocyte-vessel dissociation, leading to enlargement of blood vessels, together with dysregulation of signalling pathways controlling vessel diameter likely underlie disruption of neurovascular coupling in brain metastasis. Upregulation of vasoconstrictory molecules (p450/20-HETE) at the arteriole end of the vascular bed may lead to reduced basal blood flow as observed *in vivo* (see Serres et al., submitted abstract) and suppression of response to stimulation, whilst upregulation of vasodilatory mediators (iNOS, COX, GSH) downstream of the arterioles may reduce vascular reserve and, hence, vascular responses to stimulation.

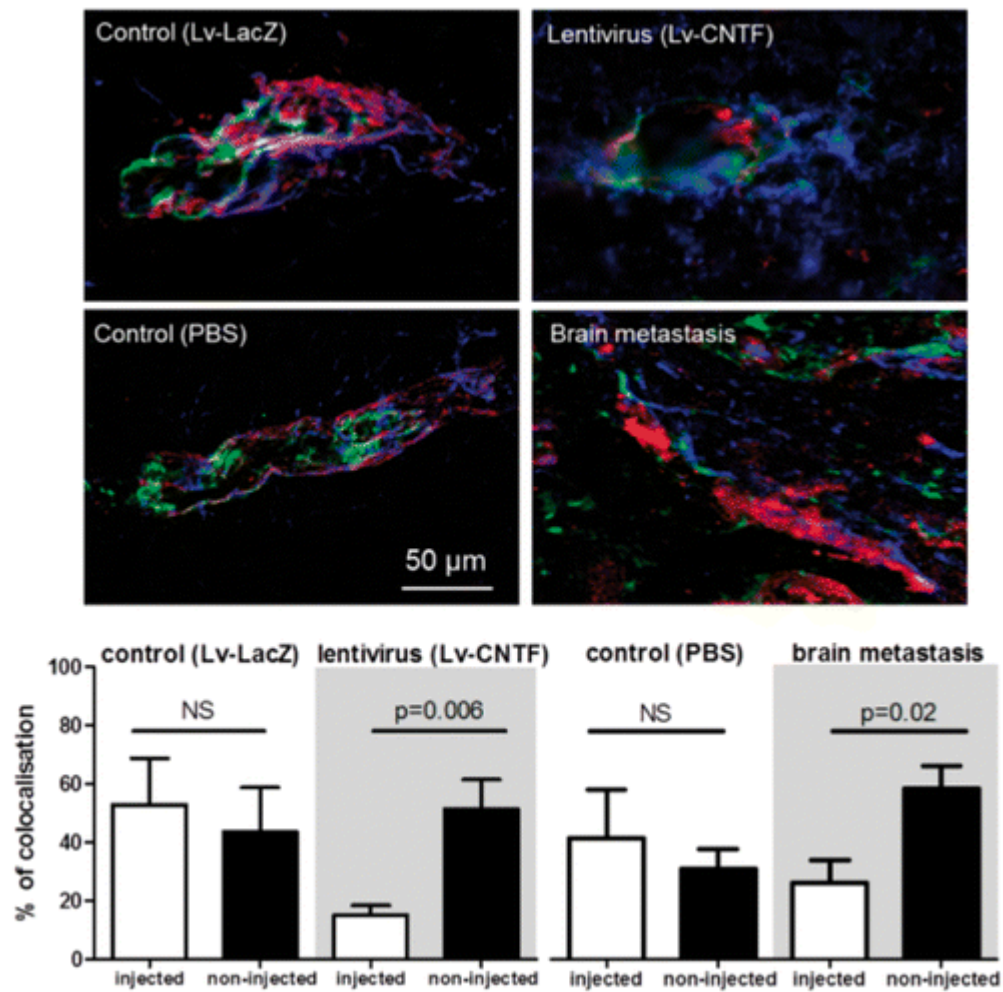


Figure 1- Co-localization of β -dystroglycan (green), astrocyte (blue) and blood vessel (red) is reduced in the area of either Lv-CNTF or brain metastasis induced astrocyte activation.