Effects of Akt overexpression on blood vessels

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Introduction

Akt (also known as PKB) is a serine/threonine protein kinase that is activated by various growth factors and cytokines, including many angiogenic factors (1, 2). Akt signaling in endothelial cells regulates multiple critical steps in angiogenesis. We have used a binary transgenic mouse model that expresses dominant active Akt (myrAkt) in endothelial cells in an inducible manner to investigate the effects of sustained endothelial Akt activation on vascular structure and function by MRI and histology.

Materials and Methods

tTA:VE-cadherin x TET:myrAkt double transgenic mice were generated by crossing TET:myrAkt mice with tTA:VE-cadherin mice. In these mice, myrAkt was expressed only in endothelial cells, and expression was suppressed by addition of tetracycline in the drinking water. myrAkt expression was induced for 7 days prior to MRI scan by withdrawal of tetracycline. Dynamic contrast enhanced MRI using biotin-BSA-GdDTPA was done as reported previously (3) on a 4.7T Bruker Biospec spectrometer. Precontrast R1 was determined by non-linear fit using 3D-GE variable flip angle data (3). R1 weighted 3D-GE data (TE=3.561ms; TR=10ms; 128x128x64 with FOV of 12x12x6cm; acquisition time per image163sec; total followup after administration of the contrast material 30 min) was analyzed for derivation of the blood volume fraction (fBV) and vascular permeability (PS) as reported previously (4). The blood volume fraction (fBV) in the tissue was determined as the ratio of biotin-BSA-GdDTPA concentration extrapolated to the time of contrast material administration to the concentration of the contrast material in blood (as measured in the vena cava). Following MRI analysis, tissues were retrieved for histological staining of the biotinylated contrast material with avidin-FITC (5).

Results

Blood volume: Blood volume fraction (fBV) was higher in all organs of the myrAkt expressing animals. Increase in fBV was statistically significant in the brain and hind limb muscles in myrAkt animals as compared to control animals (brain: $p=0.005$, limb: $p=0.003$; 2tail unpaired t-test; control: n=6, experiment: n=6).
The systemic increase in blood volume was manifested by the dilution of the contrast material in the circulation as measured in the vena cava at time zero (Figure 1). Although the same amount of contrast material was administered intravenously, the concentration of biotin-BSA-GdDTPA in the vena cava in myrAkt mice was significantly lower than in control mice ($p<0.05$; 2tail unpaired t-test; control: n=6, experiment: n=6).

Vascular permeability: The extravasation rate (indicated by PS - Permeability Surface Area Product), was calculated as the rate of contrast accumulation during the first 5 scans (~15min) post contrast agent administration, scaled by the concentration of contrast material in blood as determined from the vena cava. A trend of higher permeability was seen in all organs for the myr-Akt expressing mice, but only the liver showed significantly higher permeability ($p=0.01$; 2 tail unpaired t-test; control: n=6, experiment: n=6), while in other organs: brain, hind limb and kidney, the change observed was not significant ($p>0.05$; 2tail unpaired t-test; control: n=6, experiment: n=6).

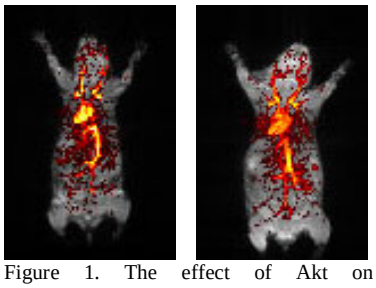

Figure 1. The effect of Akt on intravascular distribution of biotin-BSA-GdDTPA. Overlay of maximal intensity projections from pre-contrast 3D-GE images (gray) and images acquired immediately after intravenous administration of the contrast material (color). Left)- control, Right) - myrAkt double transgenic mice.

Conclusions

myrAkt expression in endothelial cells increased both blood volume and vascular permeability. These results suggest that VEGF signaling via Akt accounts for the major physiological responses observed at the early stages of angiogenesis. MRI was instrumental in analysis of the response to myrAkt expression in transgenic mice.

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