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Elucidating cannabinoids' effects on Ewing's sarcoma tumor vasculature

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Abstract

To elucidate the mechanism by which AJA affects Ewing's Sarcoma cellular pathways, we conducted an angiogenic array to observe AJA's effects on fifty-five different angiogenic proteins. The angiogenic array showed potential upregulation of TIMP-1, an angiogenic inhibitor, but similar results have yet to be replicated in subsequent ELISA's.

Solid tumors commonly have high vascular densities and increased interstitial fluid pressures (IFP), which reduce the efficacy of treatments by inhibiting the absorption of therapeutic drugs. To determine the effects of AJA and CBD on IFP, and thus on vasculature *in vivo*, we measured IFP levels in mouse xenograft ES tumors. AJA and CBD both produced significant decreases in IFP within thirty minutes of injection, affirming their potential as legitimate cancer treatments.

Background

Ewing's sarcoma (ES) is the second most common pediatric bone cancer. With patients having a 5-year survival rate of 30%, alternative treatments must be developed. Certain cannabinoids have been shown to induce apoptosis and inhibit angiogenesis in ES cells/tumors. We are specifically observing naturally occurring cannabidiol (CBD) and ajulemic acid (AJA), a synthetic cannabinoid. AJA is structurally similar to tetrahydrocannabinol (THC), the active compound in marijuana. However, AJA and CBD do not produce any psychoactive effects, making them viable treatments for children.

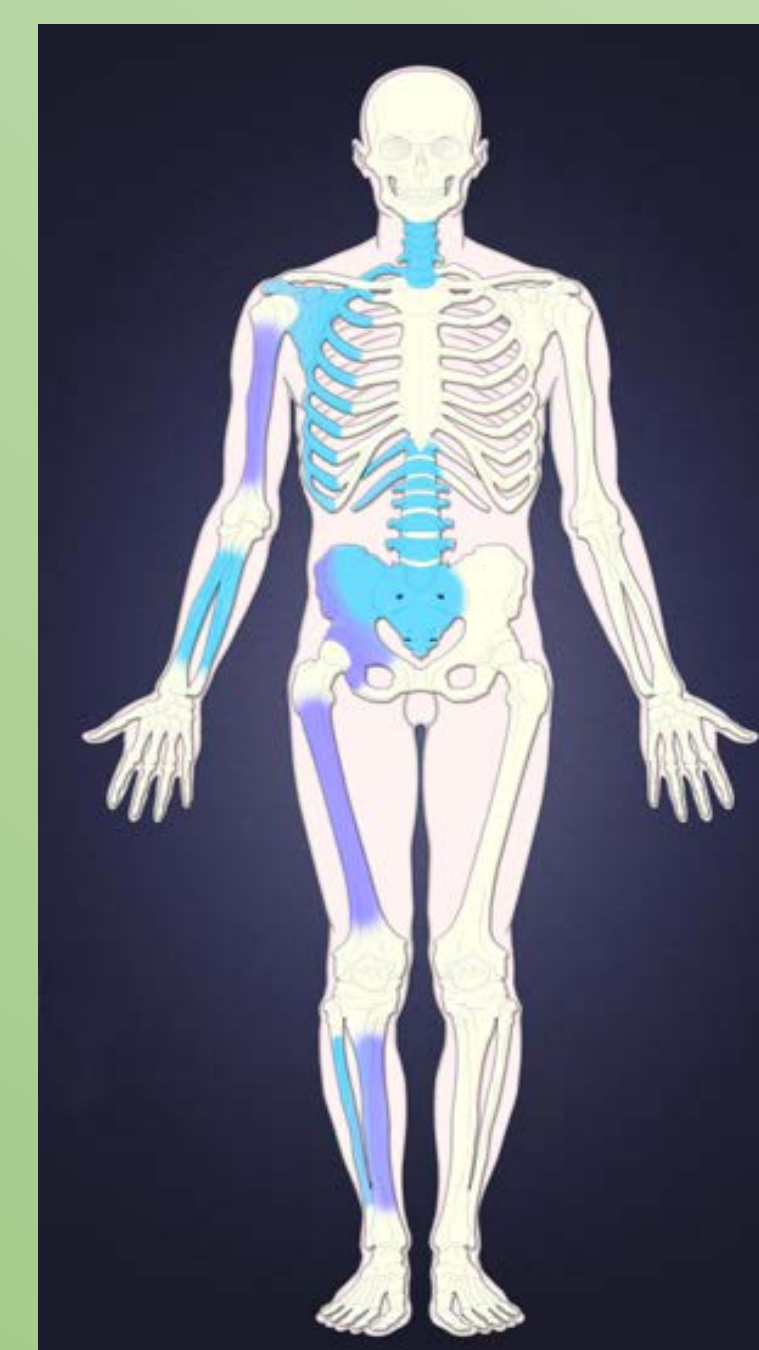


Fig. 1 – Common ES tumor locations

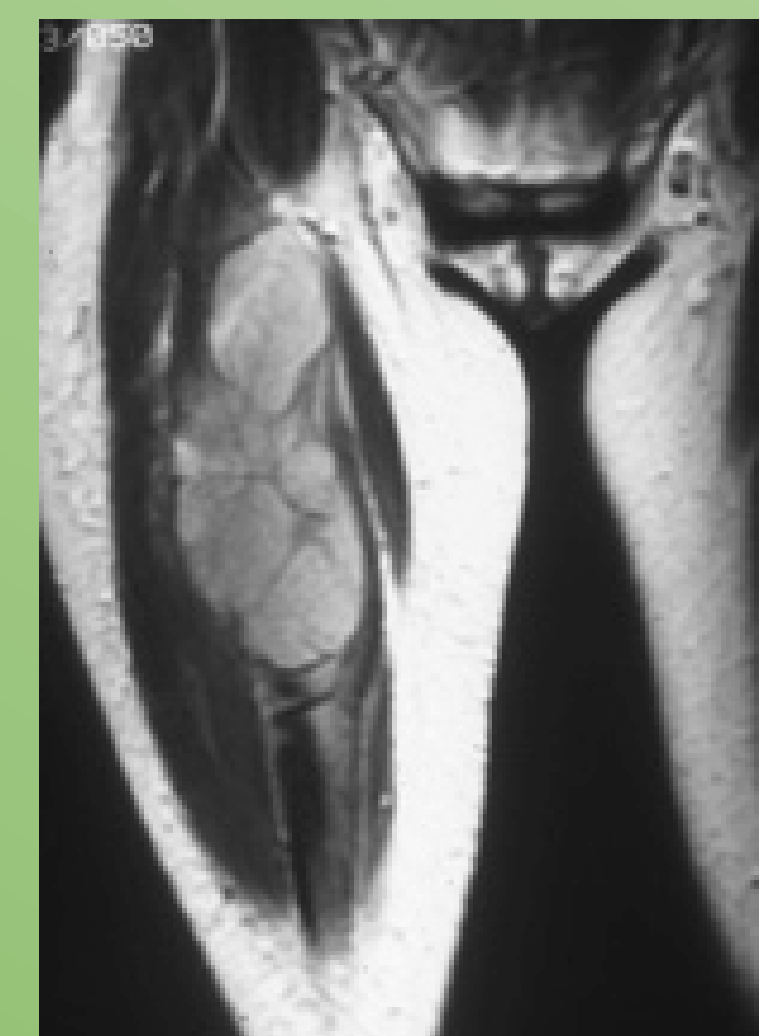


Fig. 2 – ES tumor in femur

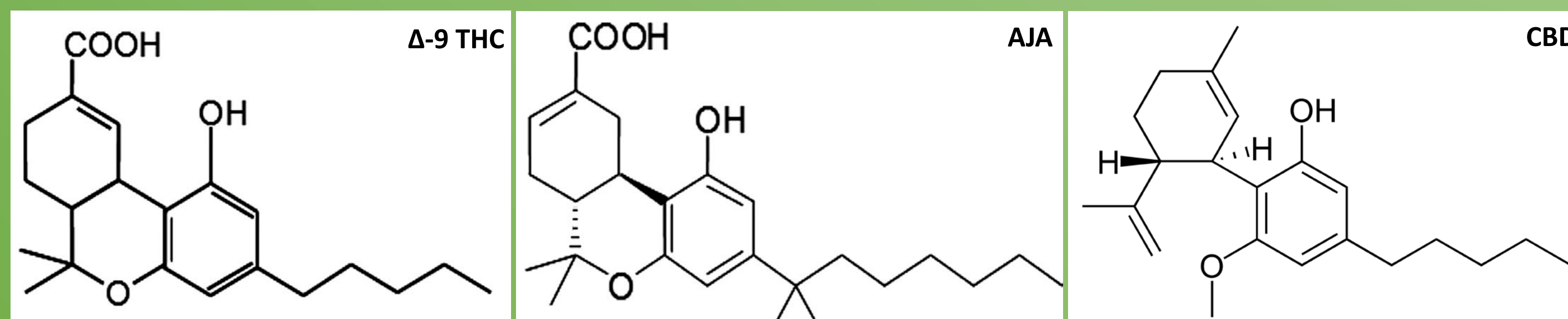


Fig. 3 – Cannabinoid structures

Previous Experiments

Aortic Ring Assays showed cannabinoids' ability to suppress angiogenesis. However, subsequent ELISA's show cannabinoids produce no consistent effects on common angiogenic proteins.

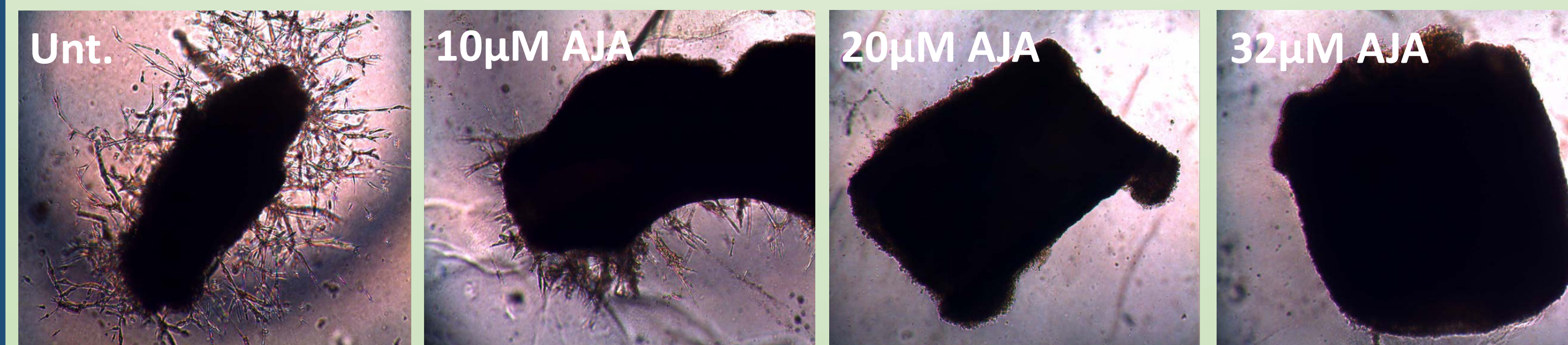


Fig. 4 - 1mm cross-sections of mouse aortas plated in matrigel, treated with indicated concentrations of AJA.

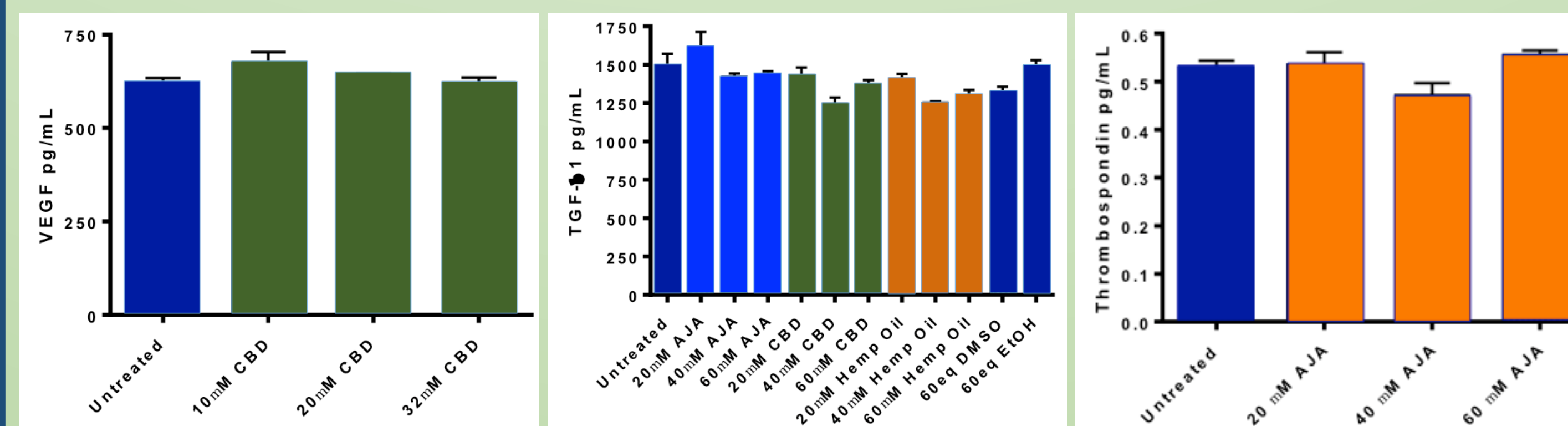


Fig. 5-7 – ELISA's for VEGF, TGF-β1, and thrombospondin1

Experiments & Results

Angiogenic Array: quantifying the effect of AJA on 55 various angiogenic proteins via antibody binding and chemiluminescence

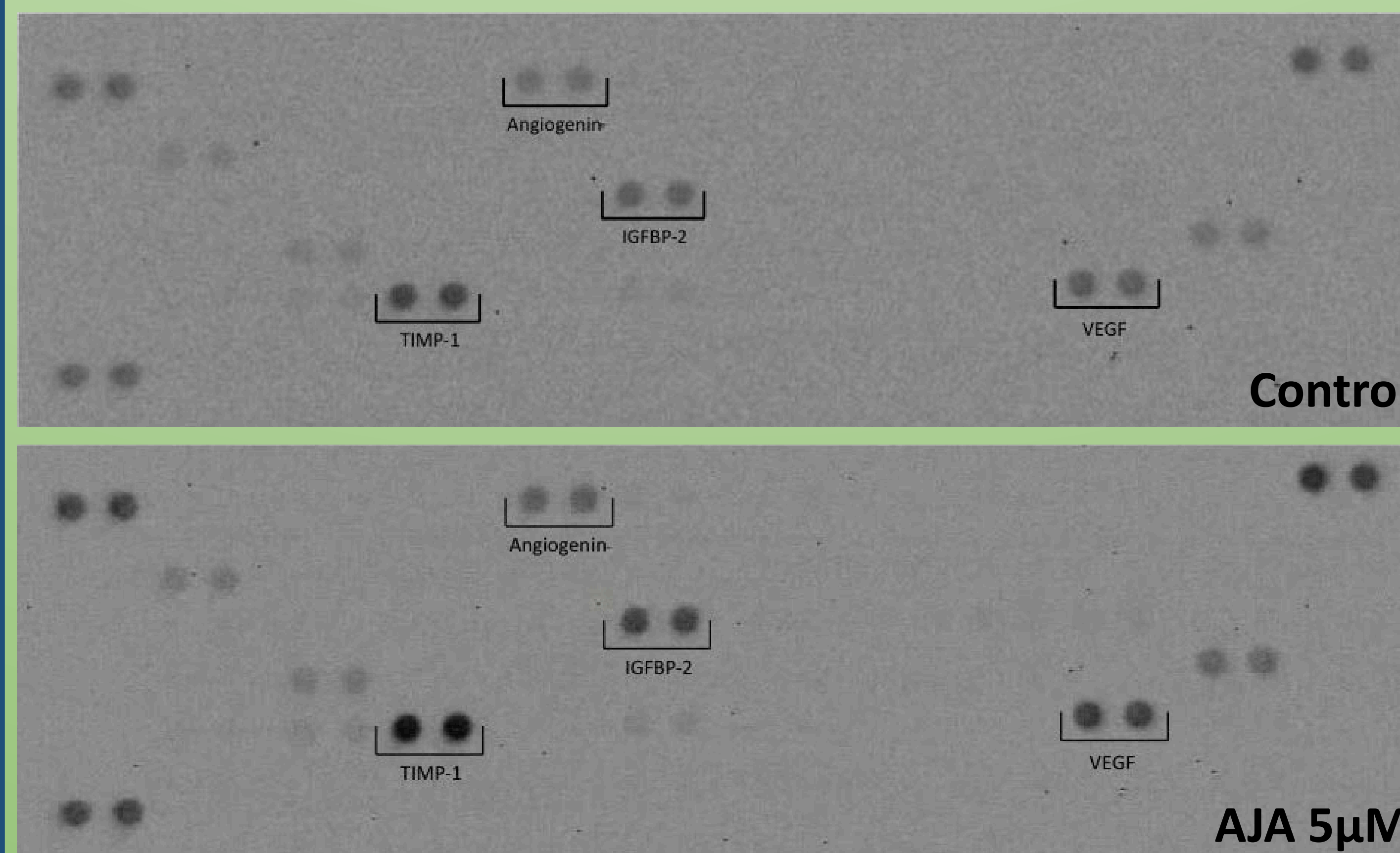


Fig. 8 – Angiogenic Array membrane luminescing

Results/Conclusion: TIMP-1, a matrix metalloproteinase inhibitor, was dramatically up-regulated upon treatment with AJA. TIMP-1 inhibits ECM degradation, making it anti-angiogenic.

Experiments (Cont.)

TIMP-1 ELISA's: quantifying effect of AJA on TIMP-1 *in vitro*

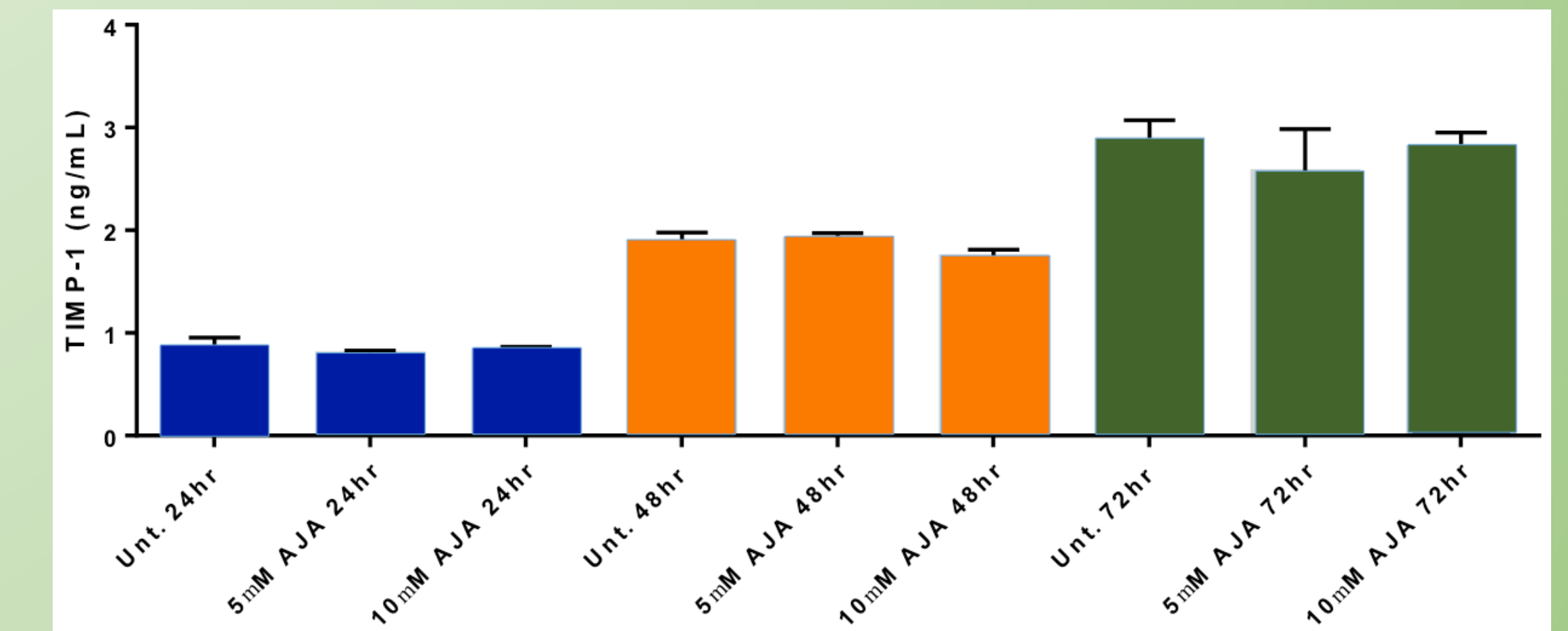


Fig. 9 – supernatant TIMP-1 levels upon AJA treatment

Results/Conclusion: AJA had no consistent effect on TIMP-1

Measuring Tumor Interstitial Fluid Pressure *in vivo*: monitoring TIFP in mouse xenograft ES tumors over 30mins. after administration of AJA and CBD (0.025mg/g)

Change in TIFP over 30mins.

Control	+7%
AJA*	-27%
CBD*	-41%

Table 1 – Change in TIFP over 30mins. *p-value < 0.05



Fig. 10 – measuring TIFP in mouse

Results/Conclusion: Mice treated with AJA and CBD displayed remarkable decreases in TIFP.

Future Studies

- Synergistic administration of cannabinoids and chemotherapy drugs
- Investigating cannabinoids' effects on angiogenic protein receptors
- Replicate spheroid invasion assay with ES cells

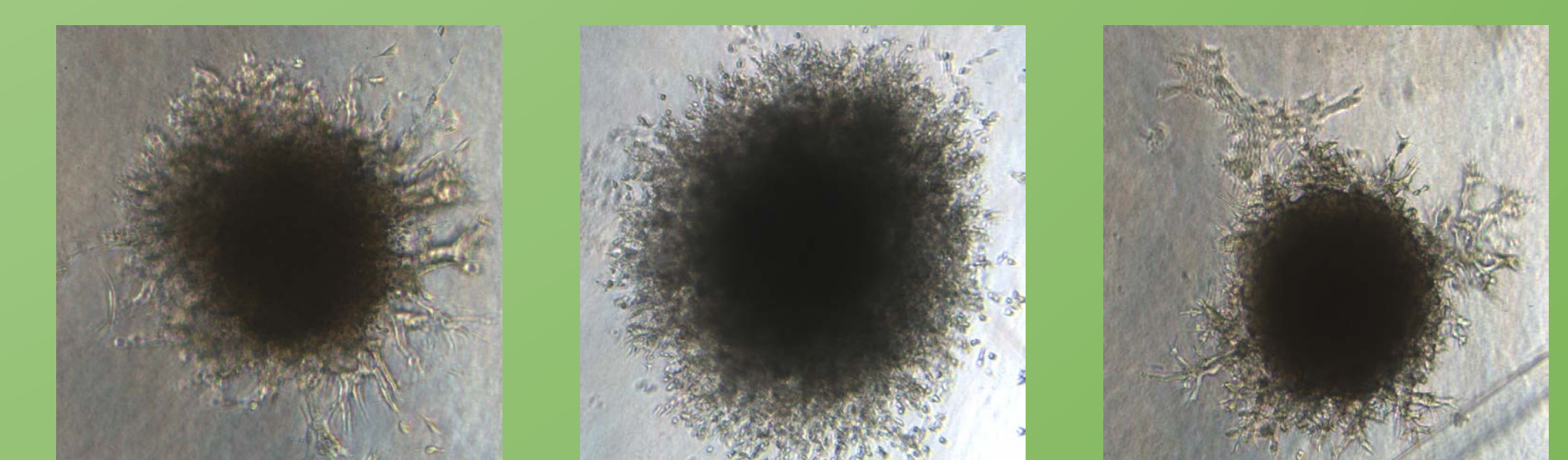


Fig. 11 – Spheroid invasion assays

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