

Characterisation of two angiosarcoma (AS) cell lines and an *in vitro* comparison of chemotherapy for the treatment of AS.

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Aim

AS is a rare but highly aggressive vascular malignancy which typically forms cutaneous disease in elderly patients. Prognosis is poor and treatment options are limited. We have obtained 2 human cutaneous AS cell lines and are currently exploring their endothelial properties. Doxorubicin (D) and Paclitaxel (P) are standard therapies in advanced AS with response rates of approximately 20%. P has anti-angiogenic properties and may be of particular benefit in AS. We therefore compared the *in vitro* response rates of D and P using an AS cell line.

Methods

Two human AS cell lines (ASM and ISO-HAS) were compared with human dermal microvascular endothelial cells (HuDMECs) as controls. Immunohistochemical characterisation of ASM and ISO-HAS was performed using a panel of endothelial cell specific markers. Expression of the angiogenic factors VEGFA, VEGFR1 and VEGFR2 were also documented. Endothelial tube formation was investigated on Matrigel. Finally response to D and P chemotherapy were explored in the ASM cell line. 10⁵ ASM cells were seeded in 1ml in 12 well plates and allowed to establish. Chemotherapy was introduced 72hours later. D and P were diluted in 0.1 % DMSO. Cells were counted at baseline, 48 and 72hours. Concentrations of 0, 1, 5, 10, 25, and 50ng/ml D and P were compared.

Results

HuDMECs, ASM and ISO-HAS demonstrated similar expression of the endothelial markers CD31, CD34 and vWF. ASM and ISO-HAS appear to over express VEGF-A and VEGFR-2 relative to HuDMEC controls. VEGFR-1 expression was minimal in all 3 cell lines. Tube formation was identified on Matrigel from 6 hours in both ISO-HAS and ASM cell lines although less vigorous than in normal HuDMECs. At low doses a cytostatic effect was observed with both D and P chemotherapy. IC₅₀ values were 24ng/ml and 12ng/ml for D and P respectively. Maximal cytotoxicity was observed at 25ng/ml for both D and P.

Conclusion

ASM and ISO-HAS are malignant cell lines which retain some normal elements of endothelial cell function. Further studies are planned to further characterise this including Boyden Chamber and Scratch Test assays. Our chemotherapy studies would suggest P as a more effective treatment in AS. There is particular interest in the role of tyrosine kinase inhibitors in the treatment of AS. Further *in vitro* studies are planned to investigate the role of the tyrosine kinase inhibitors Axitinib and Sunitinib, both as single agents and in combination with chemotherapy. This will provide interesting pre-clinical data for the Axi-STS study, a phase II study of Axitinib in soft tissue sarcoma, due to open early 2010.