

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

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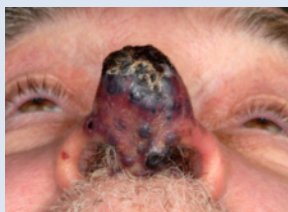
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Introduction

AS is a rare but highly aggressive vascular malignancy which typically forms cutaneous disease in elderly patients.¹ Localised disease has a tendency to recur despite wide excision and adjuvant radiotherapy. The prognosis is poor and treatment options in advanced disease are limited. We are currently exploring the hypothesis that the biology of AS is driven by aberrant angiogenic signalling.

We have obtained 2 human cutaneous AS cell lines (ASM and ISO-HAS) and are exploring their endothelial properties.



Cutaneous nasal angiosarcoma

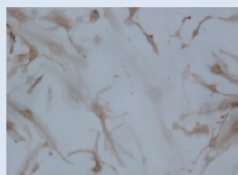
Methods

i) Immunohistochemical (IHC) characterisation of AS cell lines was performed using standard IHC protocols. Human dermal microvascular endothelial cells (HuDMECs) were used as controls. Slides were stained for CD31, CD34, von Willebrand Factor (vWF), vascular endothelial growth factor (VEGF-A) and its receptors (VEGFR-1 and VEGFR-2).

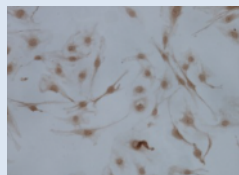
ii) Endothelial tubule formation on Matrigel was investigated. 40µl/well of Matrigel was pipetted in a 96 well plate. 15 000, 20 000 and 25 000 cells in 100µl media were added. Tubule formation was assessed at 6, 8, 12 and 24 hours for ASM, ISO-HAS and HUDMECs.

iii) 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 compared the *in vitro* response rates of D and P using an AS cell line.

100 000 ASM cells/ml were plated in each well of a 12 well plate. At 72 hours, the media was changed and chemotherapy added. Doses of 0, 1, 5, 10, 25, 50 and 100ng/ml were assessed. Cell counts were performed at 24, 48, 72 and 96 hours. 3 wells were assessed for each dose at each time point.



ASM CD31 (x20)



ISO-HAS VEGFR2 (x20)

References

1. Young RJ et al. Angiosarcoma. Lancet Oncol. 2010. (In Press).

Acknowledgements

Clinical photography - Medical Illustration Dept, Royal Hallamshire Hospital, Sheffield Teaching Hospitals NHS Trust

ASM cell line - Dr R Unger, Institute of Pathology, Johannes Gutenberg University of Mainz, Germany

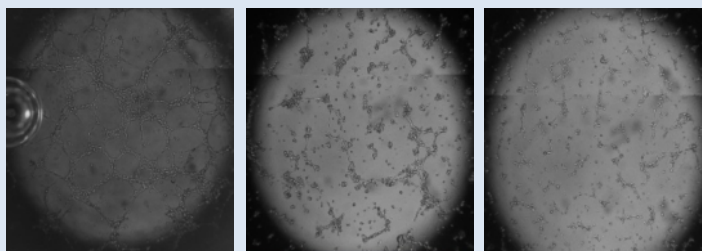
ISO-HAS cell line - Prof M Masuzawa, Cell Resource Centre for Biomedical Research, Tohoku University, Japan.

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Results

i) In keeping with their endothelial nature, both ASM and ISO-HAS stained positive for CD31, CD34 and vWF. In both ASM and ISO-HAS, intensity of staining with the endothelial specific marker CD31 was non uniform, suggesting a mixed population of cells. Diffuse VEGF-A, VEGFR-1 and VEGFR-2 staining was demonstrated in both AS cell lines and HUDMEC controls.

ii) Tubule formation on Matrigel developed with both ASM and ISO-HAS cell lines from 6 hours. However in comparison to HUDMECs tubule formation was less pronounced and required a greater number of cells seeded to initiate formation; tubule formation had also regressed by 24 hours but was persistent with HUDMECs.

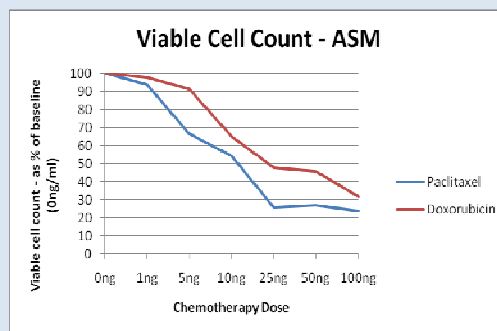


HuDMECs
15 000 cells/well at 8 hours

ASM
25 000 cells/well at 8 hours

ISO-HAS
25 000 cells/well at 8 hours

iii) Cytotoxic effects were not observed until 48 hours after the addition of chemotherapy. IC₅₀ values were 12ng/ml and 24ng/ml for P and D respectively.



Viable cell count at 96 hours post chemotherapy (ASM cell line)

Conclusion

ASM and ISO-HAS are malignant cell lines which retain some normal elements of endothelial cell function. Additional studies are planned to further characterise this including migration and invasion assays. Our chemotherapy studies would support P as a more effective treatment in cutaneous 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.

