

Analysis of Cross-talk between Human Osteosarcoma and Endothelial Cells

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INTRODUCTION

Metastasis of malignant tumour cells involves the passage of malignant cells across the vascular endothelial barrier.

We earlier reported that osteosarcoma tumour cell line (SAOS-2) induces apoptosis in cultured human umbilical vein endothelial cells (HUVEC) in a contact-dependent manner (1).

In addition, we have separately observed that SAOS-2 appears to exchange plasma membrane and cytoplasm with fibroblasts and smooth muscle cells, but does not induce apoptosis in these non-endothelial cell types (2).

AIMS

1. Confirmation of SAOS-2-induced apoptosis of endothelial cells.
2. Investigation of possible cytoplasmic and nuclear exchange between these two cell types
3. Investigation of the effect of co-culture upon cytokine synthesis, a functional property of endothelium relevant to the inflammatory response accompanying tumour development

METHODS

HUVEC isolated by collagenase perfusion were used up to passage 5 and co-cultured with SAOS-2 in 4% BSA up to 24 hours, then cell culture density determined by direct cell counting after Ulex Europaeus lectin ICC of HUVEC. DNA was isolated from cultures and run on 1% agarose gels. ELISA for IL-6, GM-CSF and G-CSF were performed for co-cultures of HUVEC and SAOS-2 in plates and transwells or after application of conditioned media.

For investigation of cytoplasmic exchange, HUVEC and SAOS-2 were also pre-labelled with CFSE and DDAO, respectively. Detection of nuclear exchange was achieved by co-culturing DDAO+Syto59 pre-labelled HUVEC and GFP expressing SAOS-2 and subsequent confocal laser scanning microscopy.

RESULTS

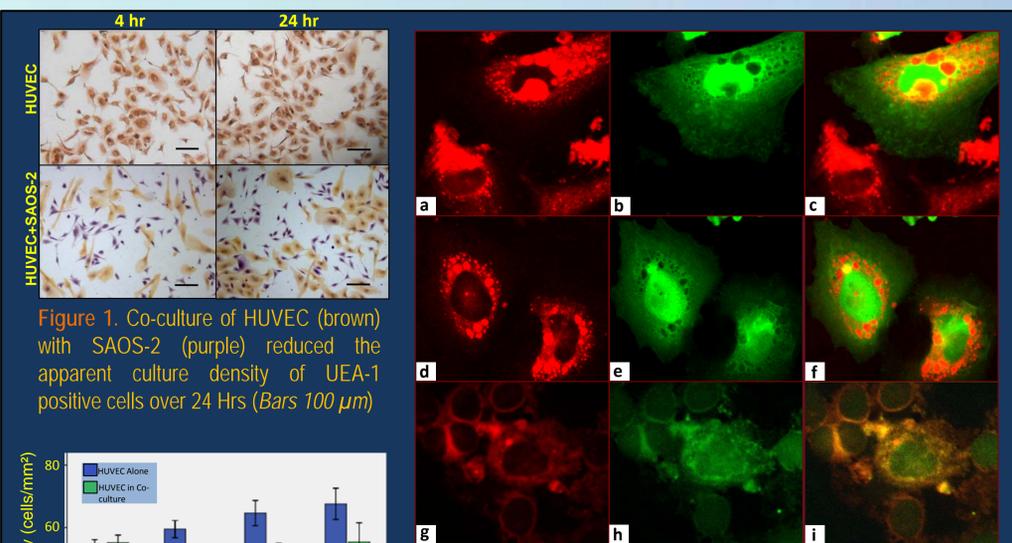


Figure 1. Co-culture of HUVEC (brown) with SAOS-2 (purple) reduced the apparent culture density of UEA-1 positive cells over 24 Hrs (Bars 100 µm)

Figure 2. In the presence of SAOS-2, HUVEC culture density decreased rapidly especially within the first 4 hours ($p < 0.001$)

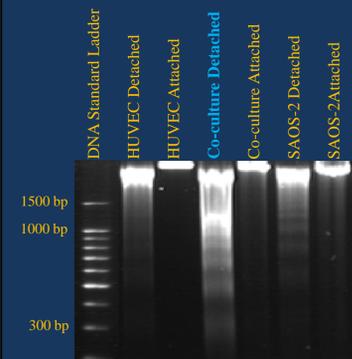


Figure 3. DNA gel electrophoresis of cultured cells. Inter-nucleosomal DNA fragmentation with characteristic band pattern formation as detected in detached co-culture.

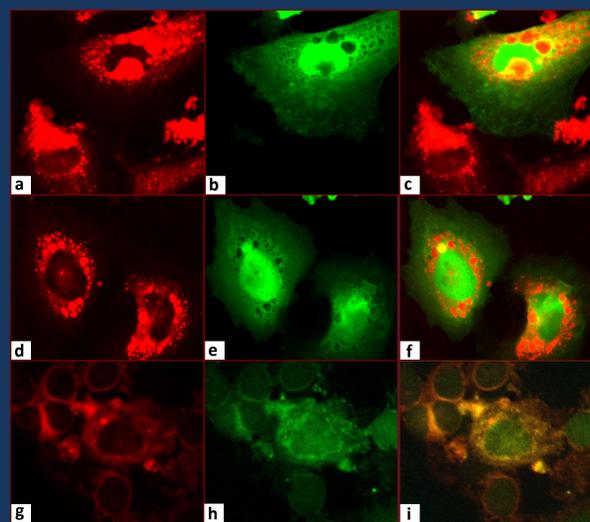


Figure 4. Photomicrographs illustrating exchange of cytoplasm and nuclear material between HUVEC and SAOS-2 in co-cultures. HUVEC pre-labelled with DDAO+Syto59 (red: a,d) or CFSE (green: b,e). SAOS-2 expressing GFP (green: b,e) and pre-labelled with DDAO (red: g). Note HUVEC internalization by SAOS-2 (yellow: c,f,i).

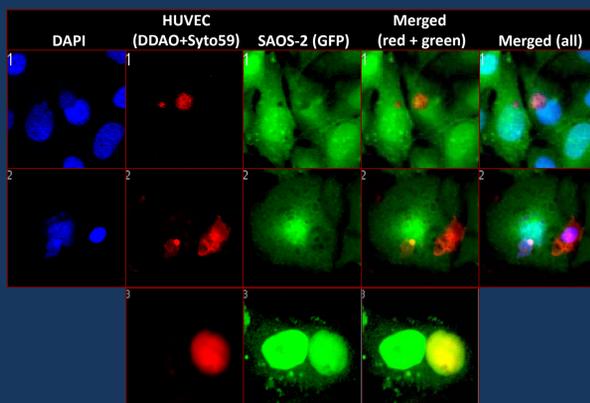


Figure 5. Photomicrographs confirming nuclear exchange between HUVEC and SAOS-2 in co-cultures. HUVEC pre-labeled with DDAO+Syto 59 (red) and SAOS-2 expressing GFP (green). Yellow in merged images indicates "fusion". Co-localization of DAPI (blue) with yellow regions confirms nuclear exchange and fusion.

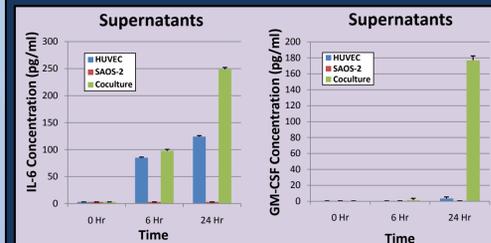


Figure 6. Graphs illustrate the concentrations of inflammatory cytokines IL-6 and GM-CSF in co-cultures. In the presence of SAOS-2, the concentrations of both IL-6 and GM-CSF increased significantly within 24 hours.

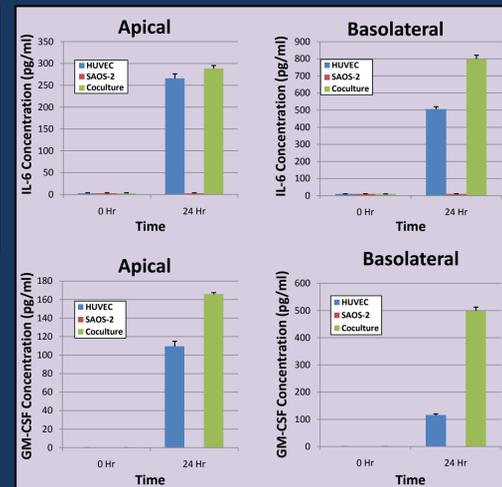


Figure 7. Graphs illustrating the concentrations of IL-6 and GM-CSF in transwell cultures separating HUVEC from SAOS-2. In co-cultures, IL-6 and GM-CSF concentrations increased significantly within 24 hours in a manner independent of contact, while HUVEC secretion was mostly basolateral in direction.

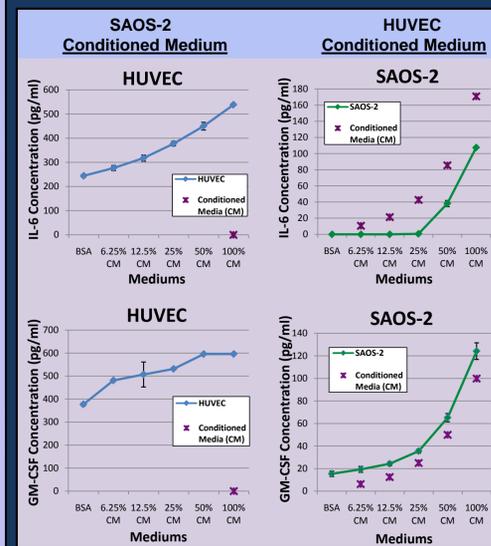


Figure 8. Graphs illustrating the concentrations of IL-6 and GM-CSF after applying conditioned medium. Note dose-dependent increase in cytokine levels due to soluble factors released by SAOS-2. In contrast to production of GM-CSF by SAOS-2, level of IL-6 decreased.

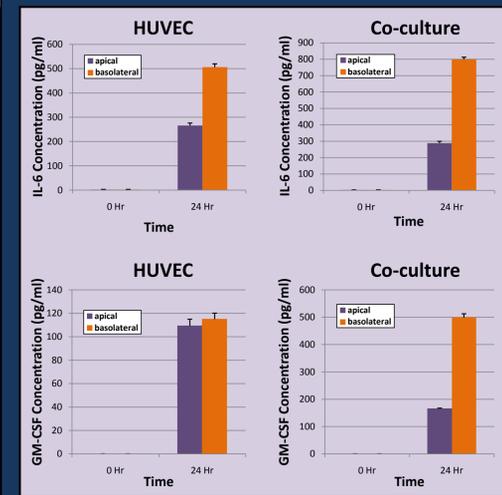


Figure 9. Graphs illustrating the concentrations of IL-6 and GM-CSF in transwell co-cultures. Elevated cytokine production was more prominent in basolateral compartments which confirms polarized secretion of IL-6 and GMCSF towards SAOS-2.

CONCLUSIONS

SAOS-2 induced apoptosis in HUVEC. In the presence of SAOS-2, HUVEC produced high amounts of inflammatory cytokines; IL-6, GM-CSF and G-CSF, in a polarized manner towards SAOS-2 suggesting a response to soluble factors released by SAOS-2. There was cytoplasmic and nuclear exchange between SAOS-2 and HUVEC indicating a potential fusion of these cells.

Osteosarcoma-endothelial cell interactions are complex ranging from cytoplasmic/nuclear exchange and apoptosis to altered cytokine synthesis with implications in microvascular remodelling, metastasis and vascular mimicry.

REFERENCES

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2. Huynh M, Zoellner H. Tumour-stromal cell fusion as a possible mechanism for invasion. *The IADR/AADR/CADR 85th General Session and Exhibition, New Orleans, LA March 24, 2007; Seq#301-Oral Cancer.*

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