An inherent connection between endothelial cell activation and miRNA biogenesis during tumour-associated angiogenesis

Matthew Warner[1], Bailey Massa[1], Jessica Haslam[1], Maria Chatzifrangkeskou[1], Tyson Sharp[2], Michael Plevin[3] & Dimitris Lagos[1]

1. Centre for Immunology & Infection, University of York, York; 2. Barts Cancer Institute, Queen Mary University, London;
3. Department of Biology, University of York, York.

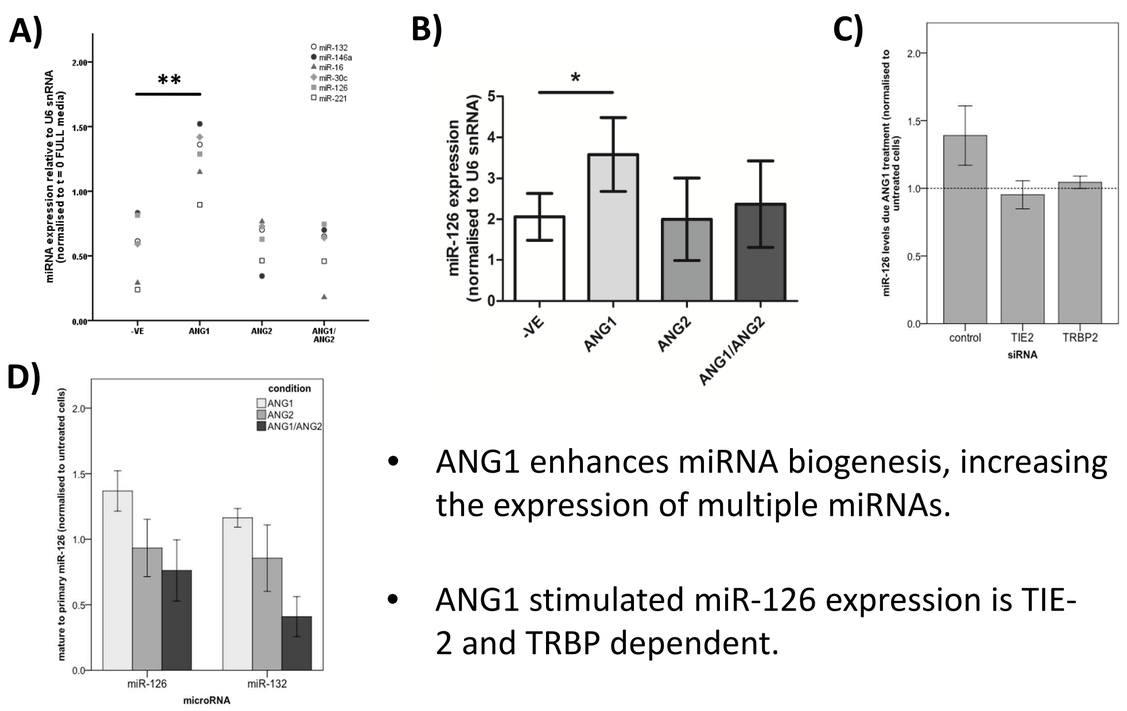
INTRODUCTION

Dysregulation of the angiopoietin (ANG) TIE-2 signalling axis is associated with tumour angiogenesis and lymphangiogenesis. Numerous studies have demonstrated that increased expression of ANG2 relative to ANG1 correlates with poor prognosis in cancer patients and ANG inhibitors are in clinical trials for the treatment of kidney, lung and ovarian carcinomas[1]. miRNAs are a class of small non-coding RNAs that have been implicated in a diverse range of cellular and pathological processes, including angiogenesis. Despite the wealth of information demonstrating the pivotal role miRNAs play in the vasculature[2-4], the effect of the ANG-TIE-2 axis on microRNA expression remains largely unexplored. Notably, components of the microRNA biogenesis machinery can be regulated by phosphorylation. Here, we show that TIE2-mediated activation of human primary lymphatic endothelial cells (HDLECs) exerts a global effect on microRNA expression by affecting the function of the cytoplasmic microRNA-processing protein assembly.

HYPOTHESIS

The ANG/TIE-2 signalling axis regulates the activity of the miRNA processing machinery to control endothelial cell function.

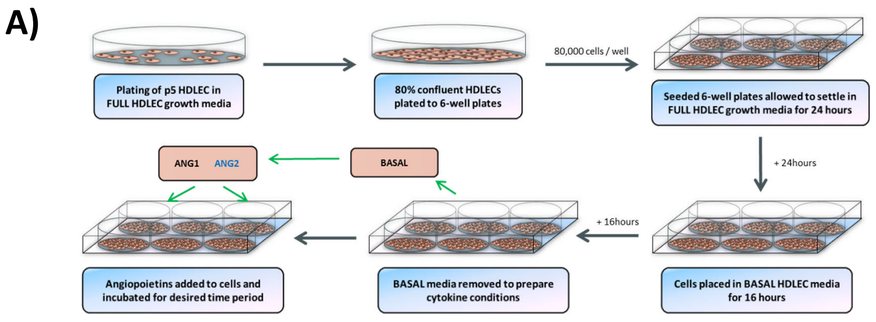
ANG-TIE-2 axis controls miRNA biogenesis in HDLECs

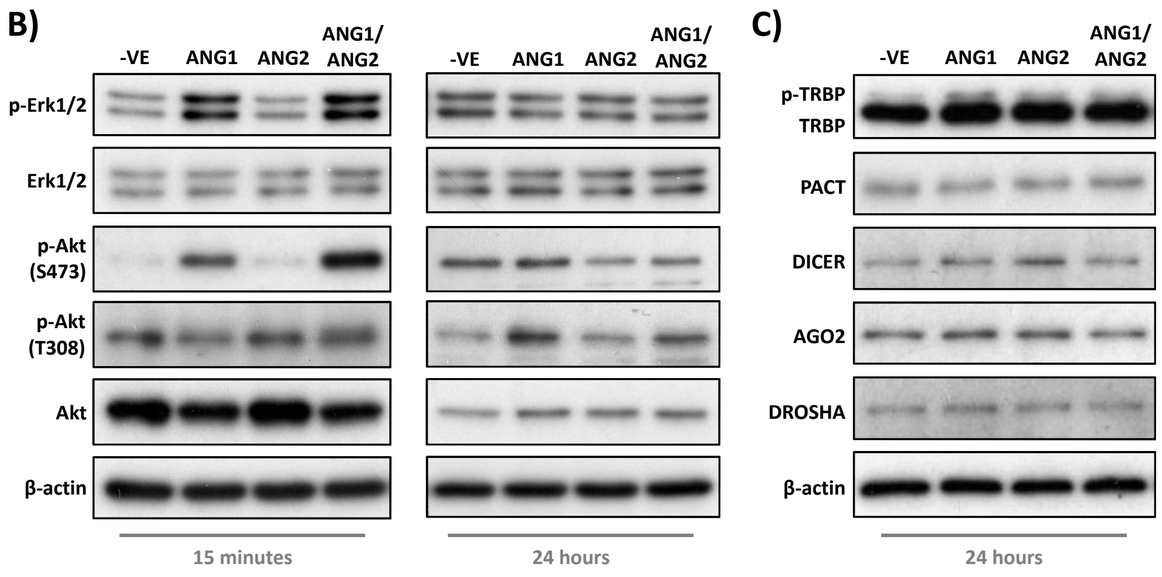


- ANG1 enhances miRNA biogenesis, increasing the expression of multiple miRNAs.
- ANG1 stimulated miR-126 expression is TIE-2 and TRBP dependent.

Levels of A) selected miRNAS and B) miR-126 after 24hr ANG treatment in HDLECs. C) miR-126 levels after 24hr ANG1 treatment and siRNA knockdown of TIE-2 and TRBP. D) mature/primary ratios for miR-126 and miR-132.

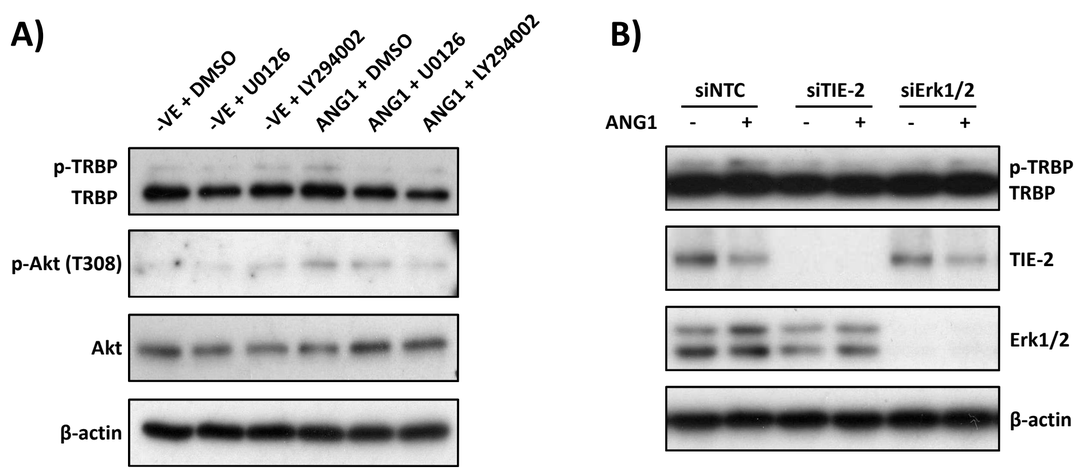
TAR RNA binding protein (TRBP) phosphorylation is regulated by angiopoietins 1 and 2





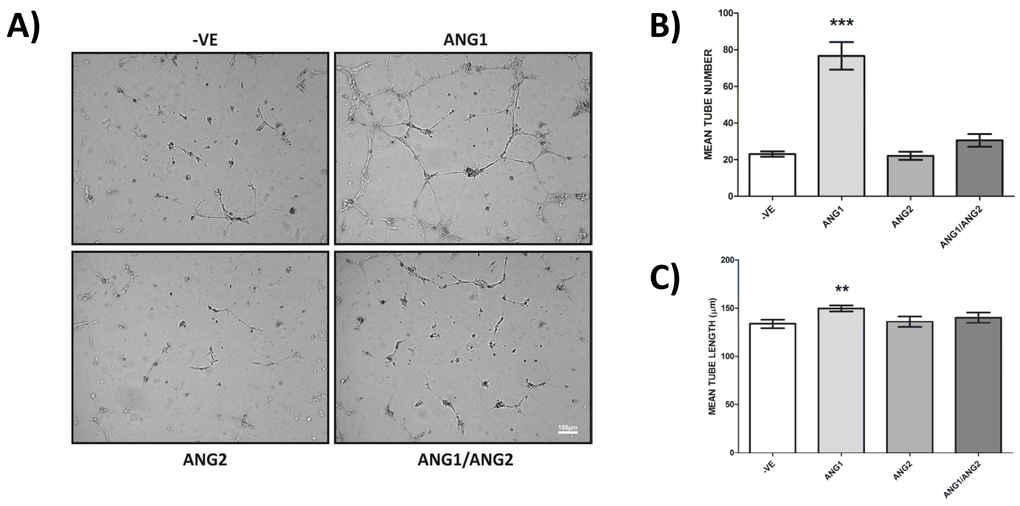
A) *In vitro* model of ANG-mediated activation of HDLECs. B) Levels of phosphorylated and total Akt & Erk in HDLECs following treatment with ANG1, ANG2, and ANG1+2 for 15min or 24hr. C) Expression levels of miRNA biogenesis machinery components in ANG1/2 treated HDLECs (24hr).

ANG1 stimulated TRBP phosphorylation is dependent on TIE-2, Erk and Akt



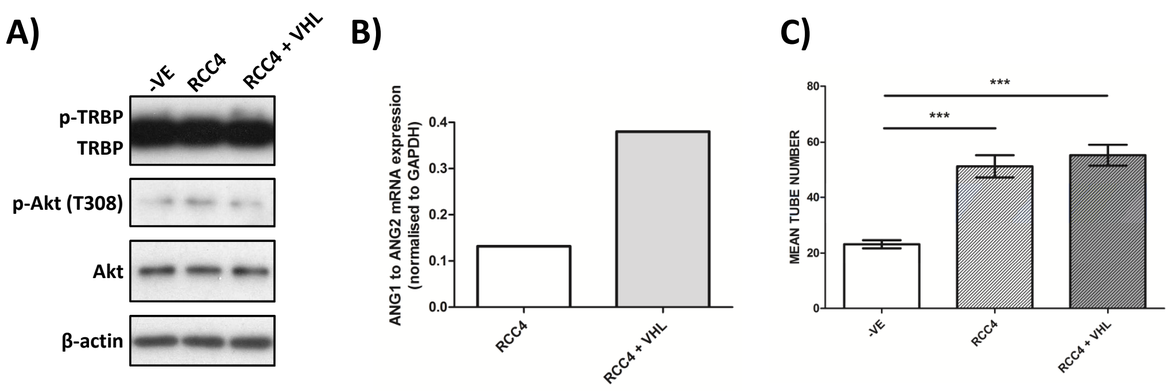
Expression of the indicated proteins following 24r ANG1 stimulation of HDLECs after A) pharmacological pre-treatment with U0126 and LY294002 and B) siRNA knockdown of TIE-2 and Erk1/2.

ANG1 stimulates tube formation in HDLECs



ANG1 stimulates HDLEC tube formation. A) Representative images following tube formation. B) HDLEC tube number and C) tube length. ** p = < 0.01; *** p = < 0.001

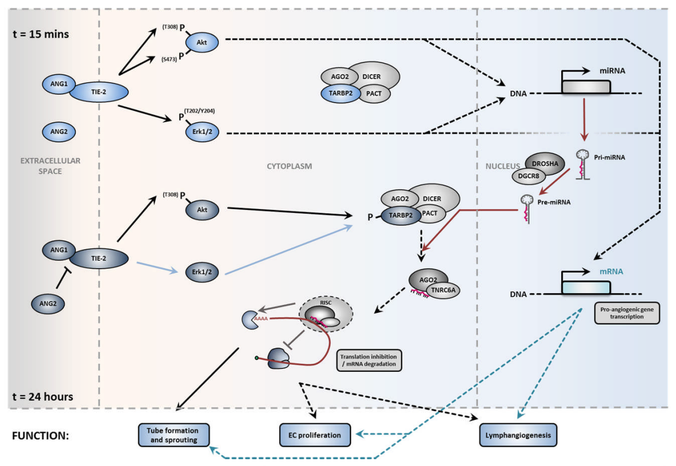
Secreted factors by renal carcinoma cells regulate TRBP phosphorylation and enhance tube formation in HDLECs



A) TRBP and Akt levels in HDLECs treated with conditioned media from RRC4 and RCC4-VHL cells and B) ANG1:ANG2 mRNA ratios in RCC4 and RCC4-VHL cells. C) HDLEC tube numbers following RCC conditioned media stimulation.

CONCLUSIONS

- The ANG-TIE-2 signalling axis regulates miRNA abundance through TRBP.
- Modulation of the miRNA biogenesis machinery is a component of the pro-angiogenic effect of TIE-2 activation.
- Our results provide clues for improving targeted cancer therapies.



TIE-2-mediated control of miRNA biogenesis as a determinant of cancer angiogenesis.

REFERENCES

1. Huang, H. *et al.* (2010) Nat Rev Cancer, **10** (8), 575-85.
2. Yang, W.J. *et al.* (2005) J Biol Chem, **280** (10), 9330-5.
3. Poliseno, L. *et al.* (2006) Blood, **108** (9), 3068-71.
4. Suarez, Y. & Cessa W.C. (2009) Circ Res, **104** (4), 442-54.