Identification of two oestrogen-sensitive microRNAs as direct inhibitors for tissue factor and factor VIII genes

Aim: High oestrogen (E_2) levels are associated with a hypercoagulable state and increased risk for venous thromboembolism, but the underlying mechanisms are not defined. We cultured HuH-7 liver carcinoma cells in the absence and presence of E_2, and identified multiple E_2-sensitive microRNAs (miRNAs) via NanoString nCounter® miRNA array, of which eight candidate miRNAs were predicted to target tissue factor (*F3*) and/or factor VIII (*F8*) genes. Therefore, the aim of this study was to investigate the direct inhibitory effects of the E_2-sensitive miRNA candidates on tissue factor and/or factor VIII gene expression.

Method: Full length-*F3* and *F8*-3'UTR sequences were cloned into the pRR5DUO dual luciferase reporter vector, co-transfected with 50nM miRNA precursors (negative control or candidate miRNAs) in HuH-7 cells, then assayed for Gaussia and firefly luciferase activities, at 24h post-transfection. To confirm direct miRNA-mRNA interactions, site-directed mutagenesis was employed to remove the putative miRNA seed sequences from the pRR5DUO-3'UTRs and assayed for miRNA-dependent inhibition of luciferase activity. Student's t-test was used to determine the statistical significance.

Result: The *F3*-3'UTR contained putative binding sites for six miRNA candidates, but only miR-365a-3p was shown to significantly inhibit *F3*-3'UTR-dependent luciferase activity ($p<0.05$). The inhibitory effect was abolished when the miR-365a-3p seed binding site was removed. Five candidate miRNAs were predicted to bind in this *F8*-3'UTR, of which only miR-548aa significantly inhibited *F8*-3'UTR-dependent luciferase activity ($p<0.05$). Deletion of the miR-548aa binding site on *F8*-3'UTR completely removed the inhibitory effects of miR-548aa on *F8*-3'UTR-dependent luciferase activity.

Conclusion: Two E_2-responsive miRNAs, miR-365a-3p and miR-548aa, were identified as novel regulators of *F3* and *F8* expression, respectively. Ongoing work is to characterise miR-365a-3p and miR-548aa regulation on F3 and F8 protein expression and function. This elucidates the E_2-miRNA regulation network contributing to E_2-associated thrombotic risk, which may be useful biomarkers for thrombosis or the development of miRNA-based therapies.