Inhibition of The Auto-inflammation Suppressor Protein ISG15 Triggers Preeclampsia by Blocking Trophoblast Migration and Invasion.

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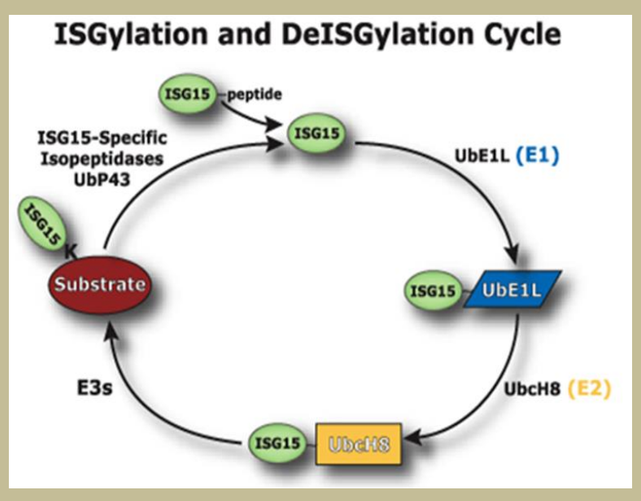
INTRODUCTION:

Interferon-stimulated gene 15 (ISG15) is a 17 kDA secreted protein encoded by ISG15 gene. The main cellular function of the protein is ISGylation (covalent binding of protein), its covalent addition to cytoplasmic and nuclear proteins, similar ubiquitination.

ISG15 shares several common properties with other ubiquitin-like molecules, but its activity is tightly regulated by specific signaling pathways that have a role in innate immunity. ISGylation by ISG-15 negatively regulates interferon (IFN) -α/β responses.

In humans, homozygous loss-of-function mutations in the ISG15 gene suppresses IFN-γ production in lymphocytes and induces an abnormally hyper-IFN-α/β mediated immune response causing severe auto-inflammation. By using microarray analysis, we found that IL-6 inhibits ISG15 expression in primary cytotrophoblast (CTB) cultures. Our *in situ* analysis showed that interstitial CTBs in preeclampsia *vs.* control placentas exhibit significantly lower ISG15 immunoreactivity.



Our hypothesis is that differential regulation of IL11-mediated gene expression by IL6 in CTBs promote decidual auto-inflammation by inhibiting ISG15, which in turn elicits the development of an inflammatory milieu in preeclampsia. Therefore, we investigate the role of ISG15 on CTB migration and invasion.

MATERIALS and METHODS:

Immunohistochemistry (IHC) performed on GA-matched control and preeclamptic decidual specimens to determine cell specific ISG15 expression (n=4).

Cultured HTR8/SVNeo cells (ATCC, human first-trimester chorionic villi explant-derived immortalized trophoblasts) were transfected with ISG15 siRNA by RNAiMAX transfection reagent according to manufacturer's instruction.

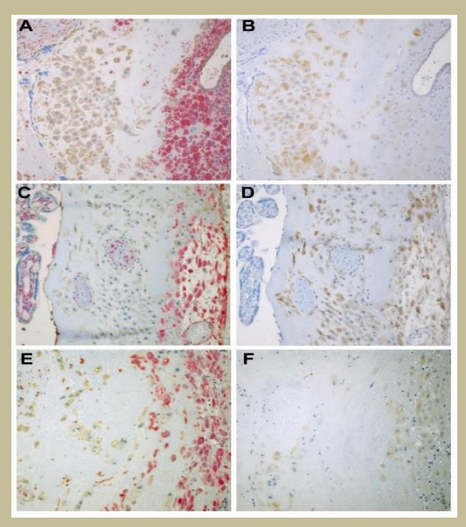
At 48h, ISG15 gene expression in HTR8 cells was detected by qRT-PCR (Taq-Man Gene Exp Assay).

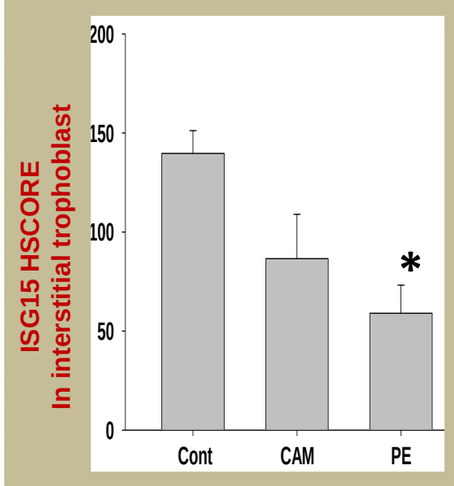
Migration and invasion assays were performed on normal and ISG15-siRNA silenced HTR8 cells using non-coated and matrigel-coated trans-wells for 24-h. Wound healing assays in 12-well culture plates were performed.

<u>Statistical Analysis:</u> Statistical analysis used student's t-test and a $p < 0.05$ considered significant.

RESULTS:

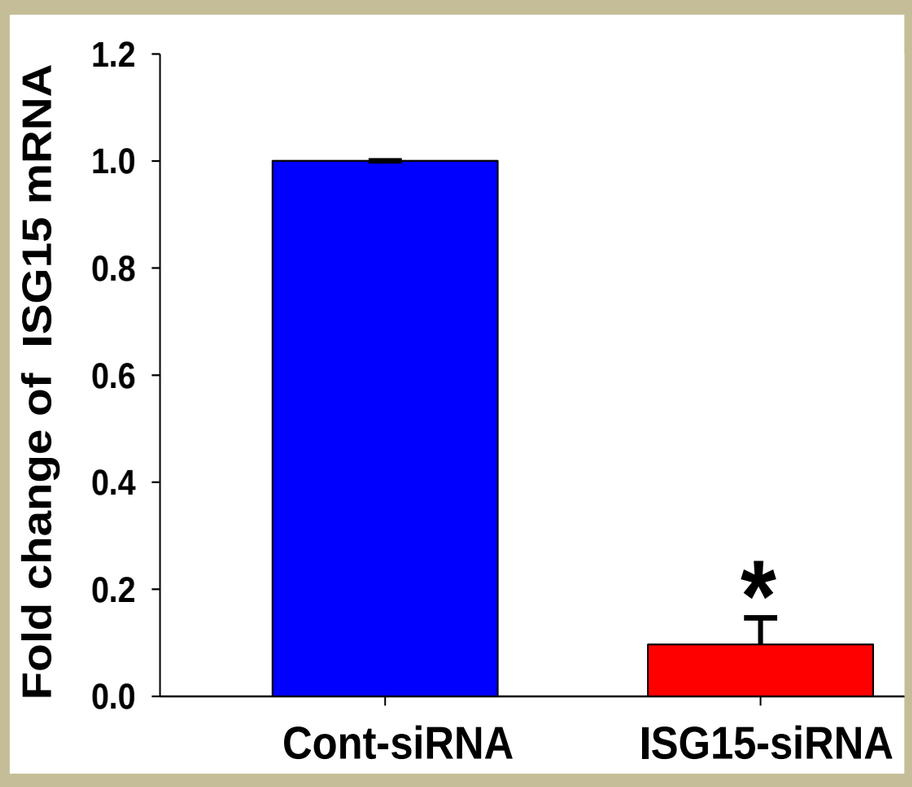
1. IHC Among decidua basalis cells, immunostaining revealed the highest in situ ISG15 expression in interstitial CTBs.



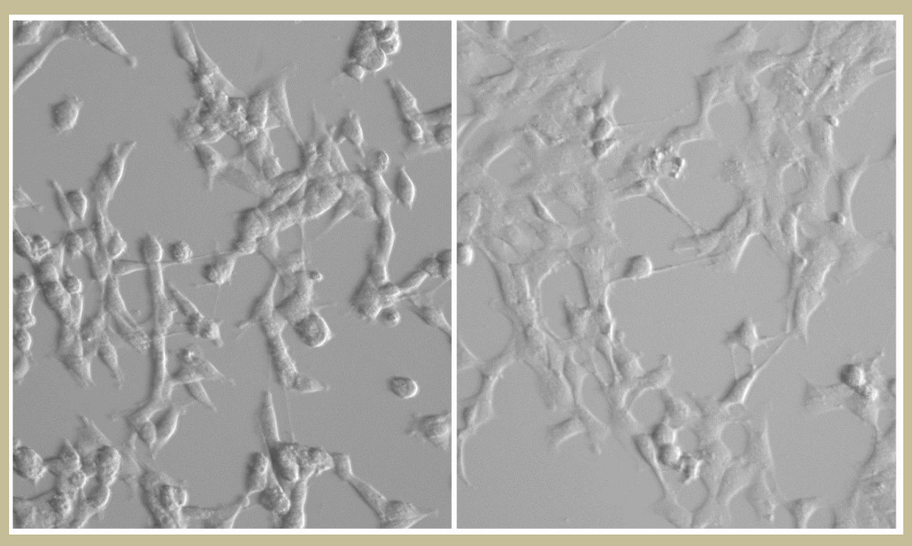


ISG15 immunoreactivity in decidua basalis. Placental specimens from gestational-age matched normal (A, B), chorioamnionitis (C, D) and preeclamptic (E, F). Cytokeratin 7 (brown) and vimentin (red) double-immunostaining of specimens was used to identify decidual cells and interstitial trophoblasts, respectively (A, C, E). ISG15 immunoreactivity in interstitial trophoblasts and decidual cells in placentae from normal (B), chorioamnionitis (D) and preeclampsia (F). HSCORE analysis of immunostaining revealed that compared to normal placentae, interstitial CTBs in preeclamptic placentae, but not in CAM associated placentae, express significantly lower ISG15 immunoreactivity (Mean ± SEM 139.7 ± 11.4, 59.0 ± 14.2 and 86.6 ± 22.4, respectively; $p<0.01$).

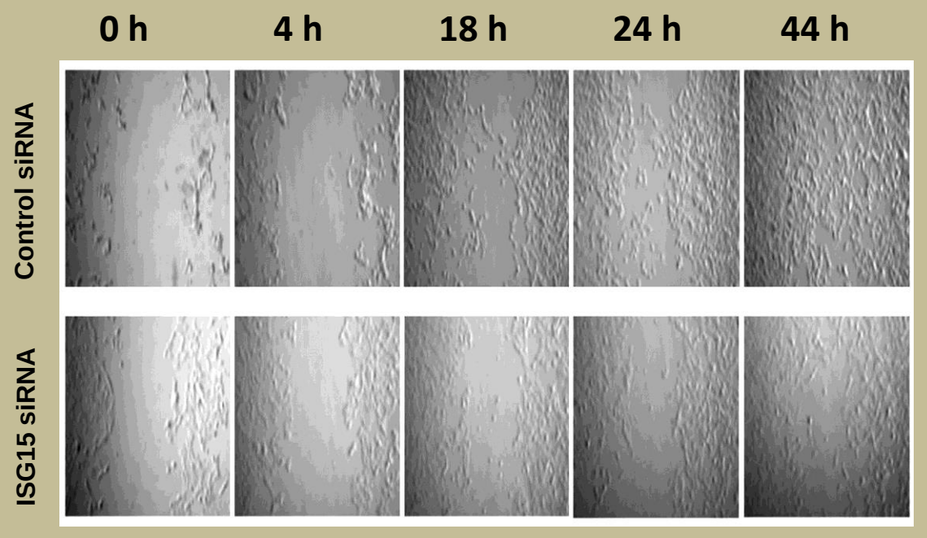
2. qRT-PCR and Immunoblot analysis of HTR8 cultures confirmed significantly reduced expression of ISG15 mRNA and protein levels following ISG15 siRNA *vs.* control siRNA treatment. (n=4 * p< 0.029 ISG15 siRNA vs Control siRNA; Mean ± SEM 0.09 ± 0.04 vs 1 ± 0.008)



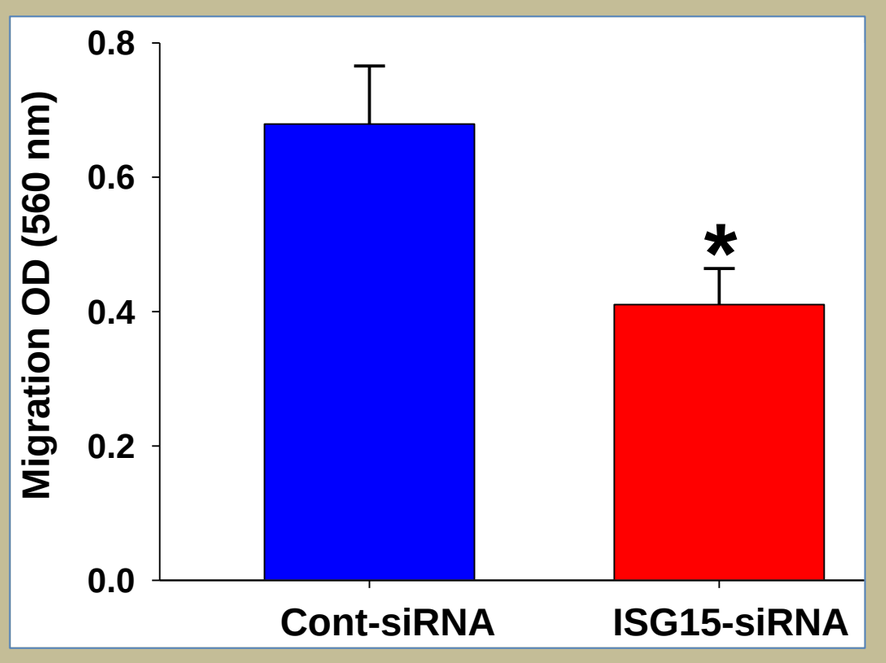
3. Silencing of ISG15 expression altered cell morphology of HTR8 cells, transiently transfected with a control siRNA (left panels) or ISG15 (right panels) siRNA.

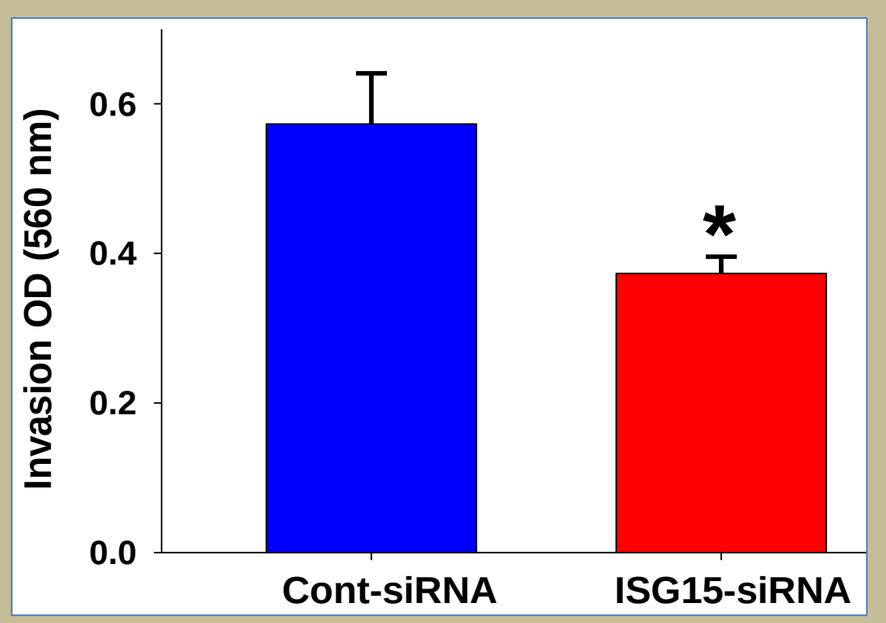


4. Silencing of ISG15 delayed *in vitro* wound healing compared to control siRNA treatment at 4 to 44 h.



5. ISG15 siRNA silencing reduced HRT8 migration (Mean ± SEM 0.410±0.05 *vs.* 0.679± 0.080; p = 0.026; n=6) and invasion (0.373±0.02 *vs.* 0.573±0.06; *p= 0.024; n=5) compared to control siRNA treatment.





CONCLUSION

ISG15 expression levels are crucial for trophoblast morphology and function (migration/invasion).

By blocking trophoblast invasion, reduced ISG15 levels could contribute to impaired spiral artery transformation that reduces utero-placental blood flow in preeclampsia. Thus, agents inducing ISG15 expression are likely to be therapeutic in preeclampsia.