Summary answer: GPRPA1 was found as a substrate of ITI-H4 to modulate the inflammatory response and was down-expressed in the sera of RPL patients.
What is known already: Thus far, the pathogenesis of RPL was not fully understood. In a previous study, the short isoform ITI-H4 cleaved by kallikrein B1 was detected in the sera of RPL patients and would be an important inflammatory factor for RPL by increasing pro-inflammatory cytokines. GPRPA1, a new binding partner of ITI-H4, was known to relate with pre-eclampsia and human decidualization by regulating angiogenesis and glycolysis. Also, GPRPA1 affects placental cell motility and cancer cell proliferation.
Study design, size, duration: Through immunoprecipitation (IP) and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF/MS) analyses, we found new binding partners of ITI-H4. Of these, GPRPA1 was selected, and direct binding between GPRPA1 and ITI-H4 was confirmed by IP and GST pull-down assay. Differential expression of GPRPA1 in sera and cellular functions of GPRPA1 in the placental cell line were investigated by molecular and cellular analyses.
Participants/materials, setting, methods: The Flag-tagged full-length ITI-H4 and the short isoform ITI-H4 were transfected into HEK293T cells and IP has proceeded with the Flag antibody. Spots showing differential expression were analyzed by MALDI-TOF/MS analysis and peptide sequence alignment was performed. The binding between GPRPA1 and ITI-H4 was confirmed using IP and GST pull-down assay. The effects of GPRPA1 on cellular functions in the placental cell were investigated by CCK-8 assay, invasion assay, and colony-forming assay.
Main results and the role of chance: Through IP, MALDI-TOF/MS analysis, and peptide sequence alignment, we found new substrates of ITI-H4 related to glycolysis, T cell activation, and production of thyroid hormones. Of these, we selected GPRPA1 which is secreted in the serum to utilize a serum biomarker of RPL. GPRPA1 directly binds to the full-length ITI-H4 and also binds to the short isoform ITI-H4 shown by IP and GST pull-down assay. Besides, GPRPA1 as a protein kinase increases serine phosphorylation of ITI-H4 and inhibits the cleavage by KLKB1. GPRPA1 is expressed significantly lower in the sera of PRL patients than the control group and knockdown of GPRPA1 negatively regulates cell motility in the placental cell. Therefore, down-expressed GPRPA1 would be one of the causes of RPL and can be utilized as a serum biomarker of RPL.
Limitations, reasons for caution: Additional *in vivo* study is needed to specifically investigate the effect of GPRPA1 on the pathogenesis of RPL.
Wider implications of the findings: By investigating the cellular functions of GPRPA1 in the placental cell, we found that it is an important key factor for the pathogenesis of RPL, and down-regulation of GPRPA1 can be utilized as a biomarker of RPL.
Trial registration number: not applicable

P-421 Down-expression of glycolytic pathway-related protein A1 is associated with the pathogenesis of recurrent pregnancy loss

H.B. Park[1], C.Z. Pei[1], H.A. Do[1], S.H. Kim[1], S.S. Park[1], B.C. Choi[2], I.K. Oh[2], Y.M. Kim[2], K.H. Baek[1]

[1]CHA university, Biomedical Science, Seongnam Gyeonggi-Do, Korea- South ; [2]Creation and Love Women's Hospital, Obstetrics and Gynecology, Gwangju, Korea- South

Study question: How does glycolytic pathway-related protein A1 (GPRPA1) relate to the pathogenesis of recurrent pregnancy loss (RPL)?