Identification of ISG15 Conjugated Proteins in Murine Reproductive Tissues During Early Pregnancy

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Interferon stimulated gene 15 (ISG15) is an evolutionarily conserved ubiquitin homolog. It becomes up-regulated in the uterus of most mammalian species in response to the implanting embryo despite significant species differences in the processes of implantation and placentation. Based on studies conducted primarily in virally-infected cells, ISG15 functions intracellularly by becoming covalently tethered to targeted conjugate proteins to modulate their stability and/or activity. ISG15 is also secreted through an unknown mechanism where it functions as a cytokine by binding and activating LFA-1 integrin to promote natural killer cell and T-cell emigration and interferon-gamma secretion. We previously demonstrated that female mice carrying the *Isg15* null mutation delivered 50% fewer pups and had abnormally low numbers of uterine natural killer cells on d7-d10 of pregnancy. Tight regulation of ISGylation through specific ubiquitin-like enzymes was also shown to be fundamentally important for normal pregnancy in that female mice deficient in the ISG15 deconjugating enzyme USP18 also had compromised pregnancies with a dramatic increase in mortality at the embryo-fetal transition. Despite these efforts to establish a fundamental role for ISGylation during early pregnancy, and despite its conservation across diverse mammalian species, the functions of this enigmatic pathway remain largely unknown in female reproductive tissues. The objectives of this study were to: 1) fully evaluate the expression of the ISG15 pathway during early murine pregnancy and human endometrial stromal cell (HESC) decidualization; and 2) begin establishing a mechanism of action through the identification of proteins that become covalently linked to ISG15 and track their fate in murine reproductive tissues. Uterine expression of ISG15, its conjugation to target proteins, and the entire ISG15 conjugation/deconjugation enzymatic pathway increased on the mesometrial side of the implantation site starting on d7 of pregnancy, with a sustained increase through d11. In parallel, ISG15 and its conjugates were elevated in the ovary on d9 of pregnancy compared to the diestrus ovary. Human *ISG15* and its conjugating/deconjugating enzymes were similarly up-regulated at the mRNA level in HESCs induced to undergo decidualization *in vitro*. Magnetic beads coated with anti-ISG15 antibody were used to immunoprecipitate ISG15 conjugates from murine mesometrial decidual tissue lysates on d9 of pregnancy, with non-pregnant (diestrus) uterine tissue used as a negative control. Isolated ISG15 conjugates were then identified using tandem mass tag-based proteomic profiling. Peptide sequences were identified from a total of 602 proteins with high z-scores based on established adjusted p-values (≤0.05). Chief among the most highly enriched decidual ISG15 conjugated proteins included those associated with interferon signaling (*e.g.*, STAT1, STAT3, JAK1, IFIT1, IFIT4, OAS3), immune cytokine signaling, signal transduction (*e.g.*, PKA and CAMK2 pathways), transcriptional regulation (*e.g.*, WNT pathway), steroidogenesis, the ubiquitin system, and cell stress. Reciprocal immunoprecipitation studies coupled with the *In Situ* Proximity Ligation Assay are being used to validate ISG15 conjugates in anticipation of future experiments that will evaluate the functional consequences of ISGylation in the uterus and corpus luteum of early pregnancy. This study was supported in part by the Curtis and Marian Rochelle Endowment in Animal Science.