Regulation of Basigin Production by Steroid Hormones and Secretion of Extracellular Vesicles in Bovine Uterine Epithelial Cells

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Basigin (BSG), a transmembrane glycoprotein, is a protein of interest due to its involvement in processes crucial to reproduction including uterine remodeling and implantation. Our objectives were to investigate steroid hormone regulation of BSG in a bovine uterine epithelial cell line (BUTE) and begin to characterize the contents of extracellular vesicles (EVs) shed by these cells. BUTE cells were treated with 0, 30, 300, and 3000 nM of progesterone (P4) 0, 30, 300, and 3000 pM of estradiol (E2), or 0, 10, 50, and 100 ng/ml insulin like growth factor (IGF-1) for 24 hours. Total protein in the cell lysates was measured via BCA. Immunoblotting of cell lysates was carried out to quantify expression of BSG. EVs from BUTE cell-conditioned medium were isolated by ultracentrifugation and characterized by transmission electron microscopy, Nano-Tracker analysis, and immunoblotting for exosome biomarkers CD09 and CD81. Immunoblotting of isolated EVs was carried out to identify the expression of basigin (BSG). BUTE cell EVs were subjected to liquid chromatography mass spectrometry (LC-MS) analysis to identify different biological pathways in which those proteins are involved. BSG expression in BUTE cells was upregulated by 300 and 3000 nM P4 and downregulated by 50 and 100 ng/ml IGF-1. However, E2 had no significant effect. This hormone-dependent regulation of BSG supports a role for BSG in bovine endometrial function. EVs from BUTE cell-conditioned medium were successfully isolated and characterized. The EVs, visualized through transmission electron microscopy, exhibited the typical cup-shaped morphology indicative of exosomes. Nano-Tracker analysis demonstrated the presence of small and large vesicles in the expected size range for exosomes (20-150 nm) and microvesicles (150-500 nm). Immunoblotting confirmed the presence of CD09, CD81, and BSG in isolated EVs. EVs were subjected to LC-MS analysis and a total of 2808 proteins were identified including CD9, CD81, basigin, semaphorin, IGFBP1, MMP-2, galectin-1, and CD44. Immunoblotting results of CD9, CD81, and BSG were consistent with LC/MS results. The identified proteins were involved in different molecular functions, biological processes, and cellular components. Some of them are translation, membrane, extracellular exosome and actin cytoskeleton organization in cellular component category and protein binding, GTP binding, and anion binding in the molecular function category respectively. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway category showed enrichment of proteasome, carbon metabolism, and nucleocytoplasmic transport. Our study provides insight into the hormonal regulation of BSG expression in BUTE cells, highlighting the impact of P4 on BSG expression in these cells. Isolation, characterization, and LC/MS analysis of proteins in EVs shed by BUTE cells opens opportunities for further investigation into the functions and significance of EVs in the bovine uterus.

Key words: Basigin, Steroid hormones, Extracellular vesicles, TEM, Nano-tracker, Western blot

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