Direct Proteomic Profiling VEGF Responsive Sulfhydromes in Human Uterine Artery Endothelial Cells

Jin Bai[1], Fenglong Jiao[2], Alejandra Garcia Salmeron[1], Olamide Fategbe[1], William Pierre Tantribeau[1], Jongwoon Kim[1], Ming Xian[3], Lan Huang[2], Dong-bao Chen[1]

1. Departments of Ob/Gyn and 2. Physiology/Biophysics, University of California Irvine, USA

3. Department of Chemical Biology, University of Brown, USA

Angiogenesis is a key mechanism that causes uterine blood flow to rise that supplies nutrients and carries out maternal-fetal exchanges of respiratory gases to support fetal growth and survival. Vascular endothelial growth factor (VEGF) is a potent angiogenic factor that stimulates human uterine artery (UA) endothelial cell (hUAEC) hydrogen sulfide (H_2S) production; yet, how VEGF stimulates uterine angiogenesis is unknown. Formations of persulfides (-SSH) from free thiols (-SH) on cysteines in proteins, referred to as S-sulfhydration, have been recently identified as the main mechanism that H_2S exerts its biological function. This study using our recently developed low-pH quantitative thiol reactivity profiling (LP-QTRP) proteomics platform hypothesized that VEGF stimulates hUAEC sulfhydrated proteins (SSH-Ps) that are involved in angiogenesis regulation. Primary hUAEC were treated with or without VEGF (10 ng/ml) for 30 min. Total hUAEC proteins were labeled by tag-switch using CN-biotin and biotinylated SSH-Ps were then quantified by immunoblotting. Total SSH-Ps in the hUAEC proteome, i.e., sulfhydrome, were labeled and trypsinized; SSH-peptides (SSH-PPs) were enriched from the tryptic peptides and then sequenced by LP-QTRP proteomics platform that can identify and quantify SSH-PPs aligned on respective SSH-Ps using LC-MS/MS. Bioinformatics tools were used to determine the biological functions of VEGF-responsive SSH-Ps and their interactomes. Levels of total SSH-Ps were significantly greater in VEGF-treated vs. untreated hUAEC. In the untreated and VEGF-treated hUAEC sulfhydromes, there were hundreds of differentially regulated sulfhydrated proteins (DRSPs) characterized by SSH-PPs that map reactive cysteine(s) in each SSH-P. Bioinformatics analyses revealed that the VEGF-responsive DRSPs are involved in regulating many key biological processes and interactomes including extracellular matrix constitution (thrombospondin, integrin and pregnancy-specific glycoprotein), cytoskeleton remodeling (vimentin), antioxidation (peroxiredoxin) and cell-cell interaction (endosialin), important for regulating uterine angiogenesis. Altogether, we concluded that VEGF stimulates protein sulfhydration in hUAEC; the unique VEGF-responsive DRSPs demonstrate that VEGF differentially regulates sulfhydration of specific proteins involved in angiogenesis regulation, informing novel roles of protein sulfhydration in uterine angiogenesis regulation by augmented endogenous H_2S production. [AHA Postdoc Fellowship 903757 (to JB) and RO1 HL70562 and RO1 HD105699 (to DBC)].