Progress Report

Prime Award No. 3 U24DK076169-07S1; Subaward No. 25732-2
"Insulin action on endothelium in angiogenesis"
Georgia Health Sciences University (Diabetic Complications Consortium Pilot and Feasibility Award)
11/01/2012-10/31/2013
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A. Specific Aims

Specific Aim 1: Determine contribution of endothelial cell insulin signaling to angiogenesis.

Specific Aim 2: Identify proangiogenic genes regulated by insulin and FoxO in endothelial cells.

The aims were not modified.

B. Studies and Results

We used Matrigel plug assays to address the hypothesis proposed in Aim 1. Matrigel plugs were implanted subcutaneously in mice with knockout of the insulin receptor targeted to endothelial cells (VENIRKO mice) or in control mice with intact insulin receptors. The animals were further divided into groups fed a high-fat or a control diet. Angiogensis in the plugs were more pronounced in control animals fed a high-fat diet but less so in VENIRKO mice fed a high-fat diet.

We used the cell culture model proposed in Aim 2 to identify endothelial cell genes regulated by insulin through FoxO1. Wildtype FoxO1 or a constitutively active FoxO1 mutant were expressed by adenovirus vectors in MS1 endothelial cells and treated with insulin (10 nM) for 4, 8, or 16 hours. Results showed that insulin regulated 3 genes (*Ctgf, Cited2, Adm*) through FoxO1. Some genes (*Klf6, Spry2, Ccnd1, Bmper, Fbn1*) were regulated by insulin, but not by FoxO1. Finally, some genes (*Tsc22d1, Id1, Hmga2, Ccrn4l, Sdpr, Meis1*) were neither regulated by insulin nor by FoxO1.

C. Significance

With these results, we have identified 3 genes robustly regulated by insulin through FoxO1 in endothelial cells. Impaired insulin regulation of these genes may be a cause of decreased angiogenesis in obesity and type 2 diabetes. These genes may be pharmaceutical targets with the purpose of improving angiogenesis in these conditions.

D. Plans

We plan to assess angiogenesis in hindlimb ischemia as described in Aim 1. We also plan to assess angiogenesis in Matrigel plugs containing siRNA targeting *Ctgf* and *Cited2*, candidate genes most robustly regulated by insulin.

We have had lower than expected fertility of mouse breeding and consequently an insufficient number of animals for use in assessing the importance of endothelial cell insulin signaling in angiogenesis, as outlined in Aim 1. Therefore, we have applied for, and received, a no-cost extension of this project until September 30, 2014.

E. Publications

1. Li Q, Park K, Li C, **Rask-Madsen C**, Mima A, Qi W, Mizutani K, Huang PL and King GL. Induction of vascular insulin resistance, endothelin-1 expression, and acceleration of atherosclerosis by the overexpression of protein kinase C beta isoform in the endothelium. Circ Res 2013;113(4):418-27. NIHMS505933.
2. Park K, Li Q, **Rask-Madsen C**, Mima A, Mizutani K, Winnay J, Maeda Y, D'Aquino K, White MF, Feener EP, King GL. Serine Phosphorylation Sites on IRS2 by Angiotensin II and PKC Activated to Induce Selective Insulin Resistance in Endothelial Cells. Mol Cell Biol. 2013;33(16):3227-41. PMC Journal - In Process.
3. **Rask-Madsen C**, Kahn CR. Tissue-specific insulin signaling, metabolic syndrome and cardiovascular disease. Arterioscler Thromb Vasc Biol 2012;32(9):2052-9 (review). PMCID: PMC3511859.
4. **Rask-Madsen C**, King GL. Vascular complications of diabetes: mechanisms of injury and protective factors. Cell Metab 2013 Jan 8;17:20-33 (review). PMCID: PMC3546345.

F. Project-Generated Resources

Protocols and data will be made available at publication of these results or within 2 years according to guidelines for this award.