Diabetic Complications Consortium

Application Title: Regulators that Mediate the Cellular Response to Chronic Hyperglycemia

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1. Project Accomplishments:

We hypothesized that the individual molecular response to glucose should be maintained in lymphoblastoid cells. In this study, we specifically tested whether lymphoblastoid cell lines could be stimulated with chronic high glucose exposure to demonstrate the differential expression, adhesion, and cellular effects found in the leukocytes of individuals with diabetic retinopathy. We attempted to address three major questions. First, was the expression of key leukocyte mediators of diabetic retinopathy inducible by high glucose in lymphoblastoid cell lines? Second, was there inter-individual differences in these changes? Third, did these changes distinguish between subjects with and without diabetic complications? Here, we report that changes found in leukocytes in the diabetic setting were inducible with high glucose in transformed lymphoblastoid cells; the response was unique to the specific lymphoblastoid cell line; it did not, though, distinguish between the different clinical groups.

Exposure of lymphoblastoid cell lines derived from different donors to high glucose demonstrated differential and heterogeneous gene expression, adhesion, and cellular effects that recapitulated features found in the diabetic state. Lymphoblastoid cells may represent a useful tool to guide an individualized understanding of the development and potential treatment of diabetic complications like retinopathy.

2. Specific Aims:

In this proposal we seek to test whether cell lines from DCCT/EDIC subjects reveal 1) intra-individual and 2) inter-individual and 3) case-control differences in gene expression with exposure to chronic hyperglycemia. We will test whether underlying genomic differences between DCCT/EDIC subjects are revealed by differences in hyperglycemia induced gene expression.

Collectively, stimulation of the lymphoblastoid cell lines with high glucose demonstrated corresponding changes on molecular, cellular and functional levels. Lymphoblastoid cell lines.up-regulated expression of a panel of genes associated with the leukocyte-mediated inflammation found in diabetic retinopathy that include: a cytokine (*IL-1B* fold change = 2.11, p-value = 0.02), an enzyme (*PKCB* fold change = 2.30, p-value = 0.01), transcription factors *(NFKB-p50* fold change = 2.05, p-value = 0.01), *(NFKB-p65* fold change = 2.82, p-value = 0.003), and an adhesion molecule (*CD18* fold change = 2.59, 0.02). Protein expression of CD18 was also increased (p-value = 2.14×10^{-5}). The lymphoblastoid cell lines demonstrated increased adhesiveness to endothelial cells ($p = 1.28 \times 10^{-5}$). Reactive oxygen species were increased ($p = 2.56 \times 10^{-6}$). Significant inter-individual variation among the lymphoblastoid cell lines in these responses was evident ($F = 18.70$, $p < 0.0001$). No significant differences between subjects in the three different clinical groups were identified.

3. Publications:

Manuscript presently under review at PLOS ONE.