R05. The Impact of Diabetic Conditions and AGE/RAGE Signaling on Cardiac Fibroblast Migration

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Diabetic individuals have an increased risk for developing cardiovascular disease due to stiffening of the left ventricle, which is thought to occur, in part, by increased AGE/RAGE signaling inducing fibroblast differentiation. Advanced glycation end products (AGEs) accumulate within the body over time, and under hyperglycemic conditions, the formation and accumulation of AGEs is accelerated. AGEs exert their effect by binding to their receptor (RAGE) and can induce myofibroblast differentiation, leading to increased cell migration. Previous studies have focused on fibroblast migration during wound healing, in which diabetic fibroblasts have been shown to be more migratory compared to healthy individuals. However, the impact of diabetic conditions as well as RAGE signaling has not been extensively studied in cardiac fibroblasts.

Therefore, the goal of this study was to determine how the AGE/RAGE signaling pathway impacts cell migration in non-diabetic and diabetic cardiac fibroblasts. Cardiac fibroblasts were isolated from non-diabetic and diabetic mice with and without functional RAGE and used to perform migration assay. Cardiac fibroblasts were plated on plastic, non-diabetic, diabetic, or diabetic collagen, and when confluence was reached, a line of migration was generated by scratching the plate and followed by treatment with pharmacological agents that modify AGE/RAGE signaling. Diabetic fibroblasts displayed an increase in migration compared to non-diabetic fibroblasts whereas inhibiting the AGE/RAGE signaling pathway resulted in a significant decrease in migration. The results indicate that the AGE/RAGE signaling cascade causes a decrease in cardiac fibroblast migration and altering the pathway will produce alterations in cardiac fibroblast migration.

**EXPERIMENTAL DESIGN & METHODS**

- **Mice**: Diabetic mice (db) are homozygous for leptin receptor (LepRb) knockout, non-diabetic mice (non-db) are heterozygous. Transgenic mice lacking an active form of RAGE (non-db RKO) were used as well as Rap1a knockout mice and their control, Rap1a WT.

- **Fibroblast Isolation**: Cardiac fibroblasts were isolated from A) diabetic and B) non-diabetic mice per isolation and maintained in DMEM containing 15% FBS.

- **Collagen Isolation**: Collagen was isolated from tails of diabetic or non-diabetic mice.

- **Drug Treatment**: AGEs (glycated albumin 0.5mg/ml), U0126 (5µM, inhibitor of ERK), and PKCζ (Pseudosubstrate inhibitors 1ug/mL; ps-PKCζ).

**Statistical Analysis**: A Student’s t-test, one-way ANOVA, or a two-way ANOVA were conducted, and ANOVA’s were followed by an appropriate post hoc.

**RESULTS**

**Figure 1**: The presence of RAGE signaling negatively impacts cardiac fibroblast migration. Cardiac fibroblasts were isolated from A) non-diabetic and B) diabetic mice, with and without RAGE (mice), and C) diabetic with and without RAGE mice, and D) Rap1a WT and KO cardiac fibroblasts plated on diabetic collagen were treated with U0126 (ERK inhibitor; 5µM) and ps-PKζ inhibitors. Inhibition of AGE/RAGE signaling competes against RAGE, which increase migration in diabetic fibroblasts. No changes in migration were noted in RKO fibroblasts which indicates PKCζ and ERK inhibition does not impact migration independently of RAGE signaling. Data represents mean ± SEM with a *p = 0.05-1, **p = 0.01, ***p<0.001.

**Figure 2**: Collagen isolated from diabetic mouse tails has significantly more CMLs and AGEs compared to non-diabetic collagen. A) Immunofluorescence (100X; scale bar = 50 µm) images for carboxymethyl lysine (CML) and advanced glycation end products (AGEs) in non-diabetic and diabetic collagen. B) Graph depicting semi-quantification of CML and AGEs present in non-diabetic and diabetic collagen. A two-way ANOVA followed by a Bonferroni post hoc test was used to determine significance (p<0.05, **p<0.01, ***p<0.001).

**Figure 3**: The elevated levels of AGEs in diabetic collagen did not significantly impact fibroblast migration. Cardiac fibroblasts isolated from A) non-diabetic and diabetic, B) non-diabetic and diabetic RAGE knockout, and C) Rap1a WT and KO mice were plated on either no collagen (results shown in figure 1), non-diabetic collagen, or diabetic collagen. The number of migrated cells were normalized to percent scratch area with mean ± SEM being depicted on graph. Two-way ANOVA with Sidak’s post hoc determined significance (**p<0.01, n = 5-10).

**DISCUSSION**

- AGE/RAGE signaling modified cardiac fibroblast migration
- None/Low levels of RAGE signaling resulted in high levels of migration
- “Normal” levels of RAGE signaling resulted in lowest amount of fibroblast migration
- High (above normal) levels of RAGE signaling induced increase migration which may be a result of increased fibroblast “activation”
- Rap1a is involved in fibroblast migration in conjunction with RAGE signaling

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