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

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

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ABSTRACT

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 diabetics have impaired fibroblast migration compared to healthy individuals. However, the impact of diabetic conditions as well as AGE/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 a migration assay. Cardiac fibroblasts were plated on plastic, non-diabetic, or diabetic collagen, and when confluency 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 increase 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

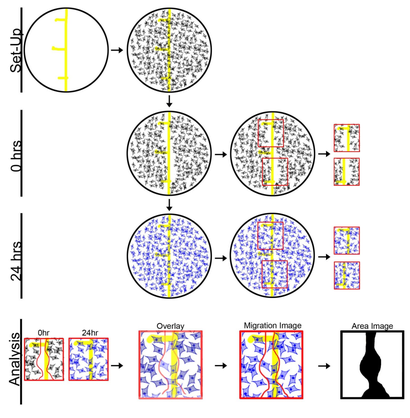
Mice: Diabetic mice (db) are homozygous for leptin receptor mutation, non-diabetic mice (non-db) are heterozygous. Transgenic mice lacking an active form of RAGE (non-db RKO) were used as well as Rap1a knockout mice and their control, Rap1a WT.

Fibroblast Isolation: Cardiac fibroblasts were isolated (2-3 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: AGE-BSA (glycated albumin 0.5mg/ml),), U0126 (5µm; inhibitor of ERK), and PKC-ζ Pseudosubstrate (1µg/mL; ps-PKC-ζ).

Migration Assay& Analysis: Used a 48 well plate with 2-3 replicates per treatment



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

Table 1: Morphological data for mice used for *in vitro* experiments. Data represents an n-value of 7-12 isolations. One-way ANOVA followed by *Dunnett's post hoc,* compared to non-diabetic (***p<0.001, ****p<0.0001)

	Body Weight (g)	Blood Glucose (mg/dL)	Heart Weight (g)
Non-diabetic (n=47)	29.05 ± 0.37	204.7 ± 7.30	0.1173 ± 0.005
Diabetic (n=28)	51.09 ± 1.26 ****	525.0 ± 22.67 ****	0.1106 ± 0.002
Non-diabetic RKO (n=41)	32.32 ± 0.43 ***	213.3 ± 4.58	0.1184 ± 0.002
Diabetic RKO (n=12)	56.61 ± 0.70 ****	412.7 ± 29.44 ****	0.1216 ± 0.002
Rap1a WT (n=34)	27.72 ± 0.35	217.2 ± 6.32	0.1106 ± 0.002
Rap1a KO (n=16)	27.61 ± 0.74	172.1 ± 6.73	0.1085 ± 0.005

HYPOTHESIS

Activation of the RAGE cascade alters cardiac fibroblast migration in type II diabetic mice.

RESULTS

1

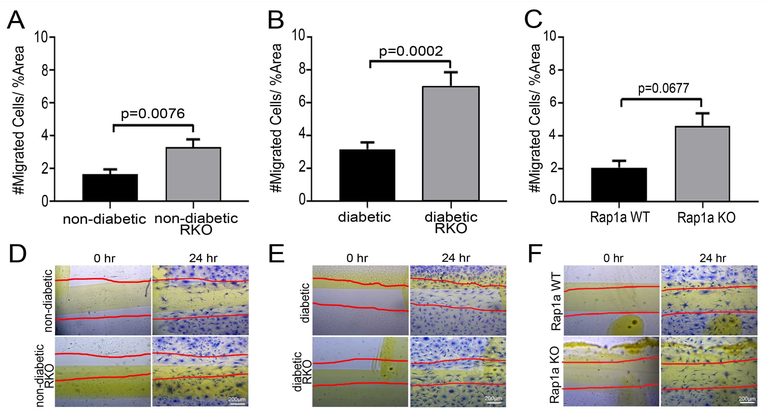


Figure 1: The presence of RAGE signaling negatively impacts cardiac fibroblast migration. Cardiac fibroblasts were isolated from (A) non-diabetic with and without RAGE mice, (B) diabetic with and without RAGE mice, and (C) Rap1a wildtype (WT) and knockout (KO) mice. (D-F) Fibroblasts were plated onto plastic cell culture dishes, scratched (0hr) and assessed for cell migration after 24 hours. Cells were stained with Coomassie for visualization with the red lines depicting scratched area (40X and scale bar = 200µm). Number of migrated cells were normalized to percent scratched area and plotted as mean ± SEM (n=7-13). Student's t-test used to determine significance.

2

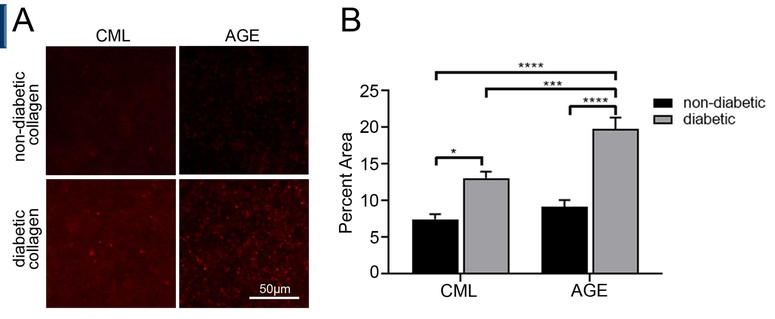


Figure 2: Collagen isolated from diabetic mouse tails have significantly more CMLs and AGEs compared to non-diabetic collagen. A) Immunofluorescence (100X; scale bar = 50 µm) images for carboxymethyl lysine (CML) and advanced glycation end products (AGEs) in non-diabetic collagen and diabetic collagen. 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* was used to determine significance (*p<0.05, ***p<0.001, ****p<0.0001)..

3

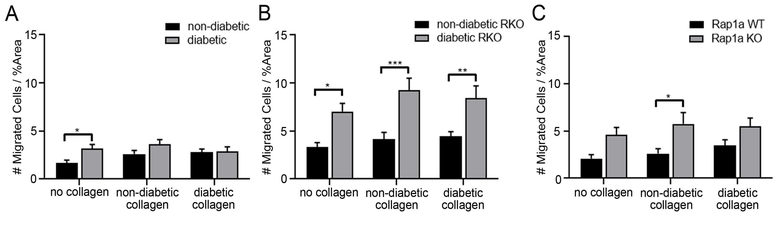


Figure 3: The elevated levels of AGEs in diabetic collagen did not significantly impact fibroblast migration. Cardiac fibroblasts isolated from A) non-diabetic and diabetic, B) non-diabetic and diabetic RAGE knockout, and C) Rap1a WT and KO mice were plated on either no collagen (results shown in figure 1), non-diabetic collagen, or diabetic collagen. The number of migrated cells were normalized to percent scratch area. Data are mean + SEM with a two-way ANOVA and a Sidak's post hoc, n = 10-13 (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001).

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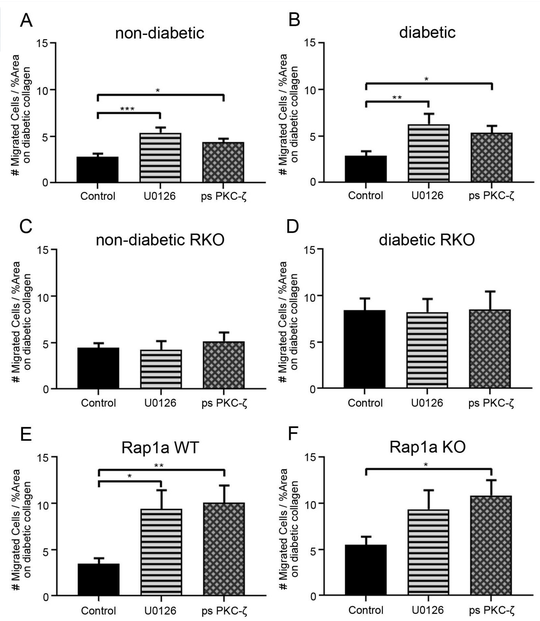

Figure 4: Cardiac fibroblasts display increased migration with decreased RAGE signaling on diabetic collagen. A) Non-diabetic, B) diabetic, C) non-diabetic RKO, D) diabetic RKO, E) Rap1a WT, and F) Rap1a KO cardiac fibroblasts plated on diabetic collagen were treated with U0126 (ERK inhibitor; 5µM) and ps PKC-ζ (pseudosubstrate PKC-ζ inhibitor; 1µg/mL). Inhibition of AGE/RAGE signaling, in RAGE signaling competent fibroblasts, led to an increase in migration. No changes in migration were noted in RKO fibroblasts which indicated PKC and ERK inhibition does not impact migration independently of RAGE signaling. Data represents mean + SEM with a n = 10-13 for control and n = 3-9 for treatments. A one-way ANOVA and a Dunnett's post hoc determined significance (* p<0.05, ** p<0.01, ***p<0.001).

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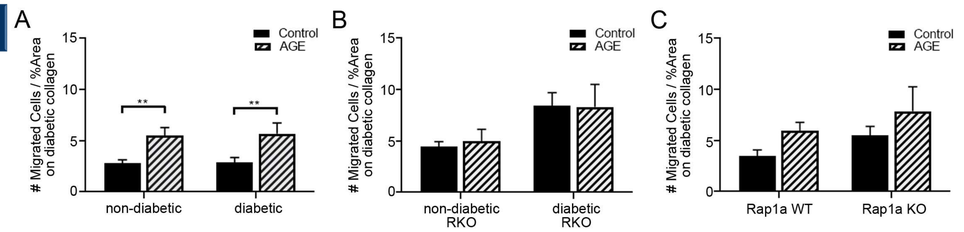
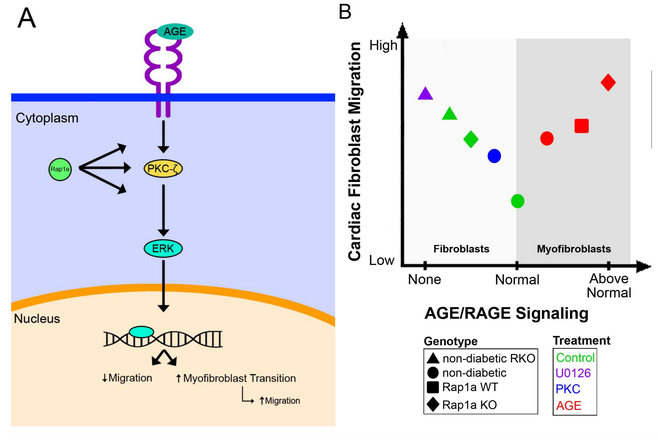


Figure 5: Increased exogenous AGEs in diabetic collagen led to increased fibroblast migration. Cardiac fibroblasts from A) non-diabetic and diabetic, B) RAGE knockout, and C) Rap1a mice were plated on diabetic collagen. Fibroblasts were either untreated (control) or treated with AGEs (0.5 mg/mL). The increased migration with AGE treatment is possibly an indirect effect. The AGE treatment may have caused an increase in "active" fibroblasts, which characteristically migrate more. Cell migration was 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 the 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

ACKNOWLEDGEMENTS

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