1. Ligand-RAGE pathway

- When the receptor for advanced glycation and products (RAGE) binds to advanced glycation and products (AGEs) or other RAGE ligands, intracellular Diaphanous is engaged, subsequently signaling pathways are activated. This results in activation of various transcription factors, such as NF-κB and Erk, which in turn activate gene expression of molecules that promote reorganization of the extracellular matrix of the vessel wall and upregulation of proinflammatory cytokines.

- This ligand-RAGE interaction drives increases in local inflammation and oxidative stress, and it is the summation of these conditions that lead to the development of diabetic and cardiovascular complications.

- The objective of this study is to investigate RAGE ectodomain shedding and the splice variants of RAGE under hyperglycemic and inflammatory conditions. Although studies have examined the regulation of RAGE shedding, little investigation into both sRAGE and esRAGE in cells exposed to stresses that provoke vascular damage in diabetes, such as hyperglycemia with the addition of a proinflammatory mediator, has yet to be explored. We hypothesized that the glucose/AGE-stimulated upregulation of inflammatory mechanisms might contribute to the generation of soluble RAGEs.

2. esRAGE and tRAGE expression is increased under inflammatory conditions

- THP-1 cells were exposed to high glucose (HG) and TNFα as a prototypic inflammatory mediator, which resulted in an increase of endogenous secreted RAGE (sRAGE) and full length RAGE (tRAGE) mRNA expression levels (fig. 2).

- As the levels of sRAGEs present by these cells were below the limit of detection in the ELISAs, we employed HEK293T cells expressing either AGER or esRAGE, which facilitated the measurement of the sRAGEs.

3. TNFα alone increases esRAGE levels, high glucose and TNFα treatment lead to increased sRAGE

- We have demonstrated using our experimental system that esRAGE levels increase under inflammatory conditions alone whereas RAGE ectodomain shedding is increased under HG and inflammatory conditions (fig. 3).

4. MMP9 and ADAM10 cause RAGE ectodomain shedding in high glucose and inflammatory conditions

- Knockdown of MMP9 or ADAM10 resulted in a >80% reduction in sRAGE levels compared to CT (control) scrambled siRNA (fig. 4A).

- This was apparent in both NG and HG HEK293T cells either untreated or exposed to TNFα.

- MMP9 mRNA expression levels increased following TNFα treatment in NG and HG conditions (fig. 4C). No change in MMP9 protein level was observed under HG or HG with TNFα treatment compared to HG conditions (data not shown). However, results showed an increase in MMP9 activity in cells exposed to HG alone (fig. 4D).

- Potentially the combined effect of TNFα increasing MMP9 levels and HG increasing ADAM10 activity levels may account for the increase in sRAGE levels under hyperglycemic and inflammatory conditions.

5. Inhibition of JNK and NF-κB decreases esRAGE mRNA levels following TNFα treatment in both normal and high glucose conditions

- A small compound screen was applied to determine which signaling pathway is regulating esRAGE expression.

- Inhibitors of ERK, AKT, JNK and NF-κB were tested. Inhibitors of ERK and AKT had little effect (data not shown), however inhibition of JNK and NF-κB resulted in a decrease in esRAGE mRNA levels (fig. 5 A).

Summary

Both forms of soluble RAGE increased in the presence of conditions that mimic diabetic complications, with the addition of TNFα treatment being a key factor. ADAM10 and MMP9 are critical to the regulation of shedding of sRAGE, with JNK and NF-κB playing an important role in the regulation of sRAGE, both in hyperglycemic and inflammatory conditions. With a better understanding of the mechanisms regulating the production of sRAGE and esRAGE under stressors there is a potential therapeutic opportunity to block adverse consequences of ligand-RAGE interactions.

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