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Fractalkine signaling in regulation of insulin secretion

Mechanisms and potential therapeutic implications?

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Fractalkine is a chemokine, which has been shown to play important roles in metabolic disease in both animal models and humans. Fractalkine is a key player in the accumulation of atherosclerotic plaques, and fractalkine receptor (CX3CR1) mutations have been implicated in obesity. Serum fractalkine levels have been found to be elevated in type 2 diabetic patients, but the role of fractalkine signaling on the pancreatic β cell was unclear. Recently published findings in April 2013 issue of the journal *Cell* by Lee and Olefsky et al. have implicated fractalkine in β-cell insulin secretion. They demonstrate that *Cx3cr1* knockout mice have impaired glucose tolerance resulting from decreased insulin secretion. In addition, fractalkine administration improved glucose tolerance and induced insulin secretion. This modulation of insulin secretion was proposed to result from an increase in intracellular calcium and potentiation of insulin secretion, which occurs in a Gαi and MEK-dependent manner. They also found that *Cx3cr1* knockout animals had transcriptional repression of genes important for β-cell function, specifically NeuroD, via induction of ICER-1. One important issue that remains unresolved is how CX3CR1 signaling regulates the potentiation of calcium influx and the distal events in insulin exocytosis. Finally, testing the effects of fractalkine treatment on proliferation and survival in vivo during regenerative conditions would be critical to determine the potential use of this chemokine in diabetes. While these exciting results open the possibility for new therapeutics, there are some concerns about a potential risk for exacerbation of atherosclerosis.

Keywords: fractalkine, CX3CL1, CX3CR1, insulin, β-cell, insulin secretion, islets, diabetes

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Fractalkine: The Pathway

Fractalkine, which is also identified as chemokine (C-X3-C motif) ligand 1 (CX3CL1) and neurotactin, is part of the chemokine family of small molecules best known for its ability to induce chemotaxis of myeloid cells.[1] Fractalkine is unique because it can exist in both a soluble and a membrane-bound form thus has the potential for long range and local tissue effects.[2] It contains a chemokine domain joined by a mucin-like stalk to the transmembrane domain that has been proposed to act as an adhesion molecule. The extracellular domain is shed from the cell surface by the ADAM (A Disintigrin and Metallopeptidase Domain) 10[3] and ADAM 17 metalloproteases and is found in the circulation in several inflammatory conditions.[4] Fractalkine is considered a pro-inflammatory chemokine, and its shedding is increased in the presence of TNF α converting enzyme (TACE). In the mouse, fractalkine is the only known ligand of the fractalkine receptor (CX3CR1), a G-protein coupled receptor. Ligand binding leads to an increase in intracellular calcium, activation of PI3K, MAPK, and Akt signaling, and actin rearrangement.[5] Fractalkine is expressed in the brain, GI tract, skin, endothelium, kidney, and lung.[6] CX3CR1 is expressed on

microglia, dendritic cells, natural killer cells, monocytes, and lymphocytes.[7,8]

Fractalkine: Role in Atherosclerosis and Metabolic Disease

Atherosclerosis

Fractalkine's importance in metabolic disease is best understood in the context of atherosclerosis initiation and progression. Fractalkine is expressed on endothelial cells[9] where it is induced in response to inflammation and plays a central role in recruiting macrophages to an atherosclerotic lesion leading to foam cell formation.[10] Fractalkine is also expressed on the surface of activated platelets which contribute to macrophage adherence to atherosclerotic plaques.[11] Humans with *CX3CR1* mutations have been found to have some degree of protection from coronary artery disease.[12]

Adipocytes

Chemokines are thought to provide a link between obesity and inflammation by participating in the recruitment of leukocytes such as macrophages to adipose tissue. Chemokine-receptor pathways implicated in adipose tissue macrophage accumulation with obesity include monocyte chemoattractant protein 1 (MCP-1) and C-C chemokine receptor 5 (CCR5).[13,14] Because of the similarities between foam cell pathophysiology and the development of adipose inflammation in obesity, the role of the fractalkine receptor in recruitment of adipose tissue macrophages has been examined. Fractalkine was found to be expressed on adipocytes and CX3CR1 on adipose tissue macrophages.[15] *Cx3cr1* knockout mice on high fat diet were shown to have increased fractalkine and CX3CR1 in epididymal fat, however, there was no difference in glucose tolerance, insulin resistance or hepatic steatosis between knockouts and controls.[15] Fractalkine/CX3CR1 signaling has also been shown to be downregulated by PPAR gamma agonists.[16,17] Thus, while fractalkine signaling may be regulated by metabolic cues, unlike atherosclerosis, the recruitment of adipose tissue macrophages with obesity is independent of fractalkine receptor signals.

Fractalkine and the β-Cell

A more expanded evaluation of fractalkine signaling in type 2 diabetes was published in the April 2013 issue of *Cell* by Lee and Olefsky et al. These studies also evaluated *Cx3cr1* deficient mice to study the effects of obesity-induced inflammation in mice with alterations in the fractalkine/CX3CR1 system and similar to other studies did not implicate CX3CR1 in regulating adipose tissue macrophage accumulation.[18] In contrast to the previous study, *Cx3cr1* knockouts were found to have glucose intolerance when on either regular chow or high fat diet. The defect in glucose homeostasis in these mice was attributed to defective insulin secretion in vivo, in vitro, and in insulinoma cell lines with *CX3CR1* silencing. This insulin secretory defect was associated with a decrease in *Pdx1*, *NeuroD*, *Ins*, *Glut2*, and *Urocortin3*. In addition, these experiments demonstrated that fractalkine treatment enhances insulin secretion by augmenting the responses to different secretagogues. This potentiation of insulin secretion was mediated in part by increasing intracellular calcium in a MEK and Gαi sensitive manner. A proposed pathway of CX3CR1 downstream signaling in the β cell is depicted in **Figure 1**. The authors conclude that the defective insulin secretion observed in the *Cx3cr1* deficient mice was mediated by ICER-1 dependent transcriptional regulation of genes involved in β-cell function and communication. However, the binding of ICER-1 to the promoter was demonstrated exclusively for NeuroD. Therefore, it would be interesting to determine the extent to which ICER-1-dependent transcription of other genes could explain the insulin secretory phenotype. In addition, how CX3CR1 signaling impacts early events in glucose metabolism and generation of ATP/ADP to regulate calcium influx was not directly evaluated. It is possible that alterations in expression of key metabolic enzymes could result in modulation of the ATP/ADP ratio and reduced inhibition of the KATP channel (**Fig. 1**). Additionally, expression of KATP/SUR channel and voltage-dependent calcium channel could also be implicated in the secretory phenotype, but there was no significant alteration in expression of these genes by mRNA (**Fig. 1**). It would be interesting to assess these genes at the protein level. Finally, the authors implied that the enhanced arginine-induced insulin secretion by fractalkine in the presence of similar intracellular calcium levels suggested a distal effect at the level of the exocytotic machinery (**Fig. 1**). The mechanisms for the distal events in insulin secretion require future investigation and are perhaps mediated by Akt signaling as demonstrated in mice overexpressing a kinase-dead Akt.[19]

The *Cx3cr1*$^{-/-}$ mice also showed interesting changes in islet morphometry. The defects in insulin secretion were accompanied by a 50% increase in β-cell area, which resulted from an increase in the number of cells that were of a reduced size. These changes are intriguing given the lack of alteration in proliferation. It is possible that the increase in β-cell area could be explained by compensation for insulin resistance. Indeed, insulin tolerance tests showed mild insulin resistance at early time points in mice exposed to high fat diet. However, further studies using more specific methods to assess insulin sensitivity could resolve this issue. The role of apoptosis in the *Cx3cr1*$^{-/-}$ was not explored, but these studies showed that fractalkine treatment induces survival and protects from palmitate-induced apoptosis. Therefore, it could be anticipated that *Cx3cr1*$^{-/-}$ mice could have decreased survival in the setting of autoimmune attack (type 1 diabetes models) or exhibit limited regenerative potential. These studies could provide additional information into the role of fractalkine/CX3CR1 system in β-cell survival and regeneration. Finally, the decreased cell size is particularly interesting because this could reflect defects in the Akt/S6 kinase pathway (**Fig. 1**), although the phosphorylation status of these molecules in *Cx3cr1*$^{-/-}$ mice was not investigated. The regulation of cell size and the activity of S6K signaling have been implicated in insulin secretion in mice with gain[20] and loss of S6K function.[21]

Another important finding from these studies was a modest alteration in insulin content observed in isolated islets from *Cx3cr1*$^{-/-}$ mice. The defect in insulin

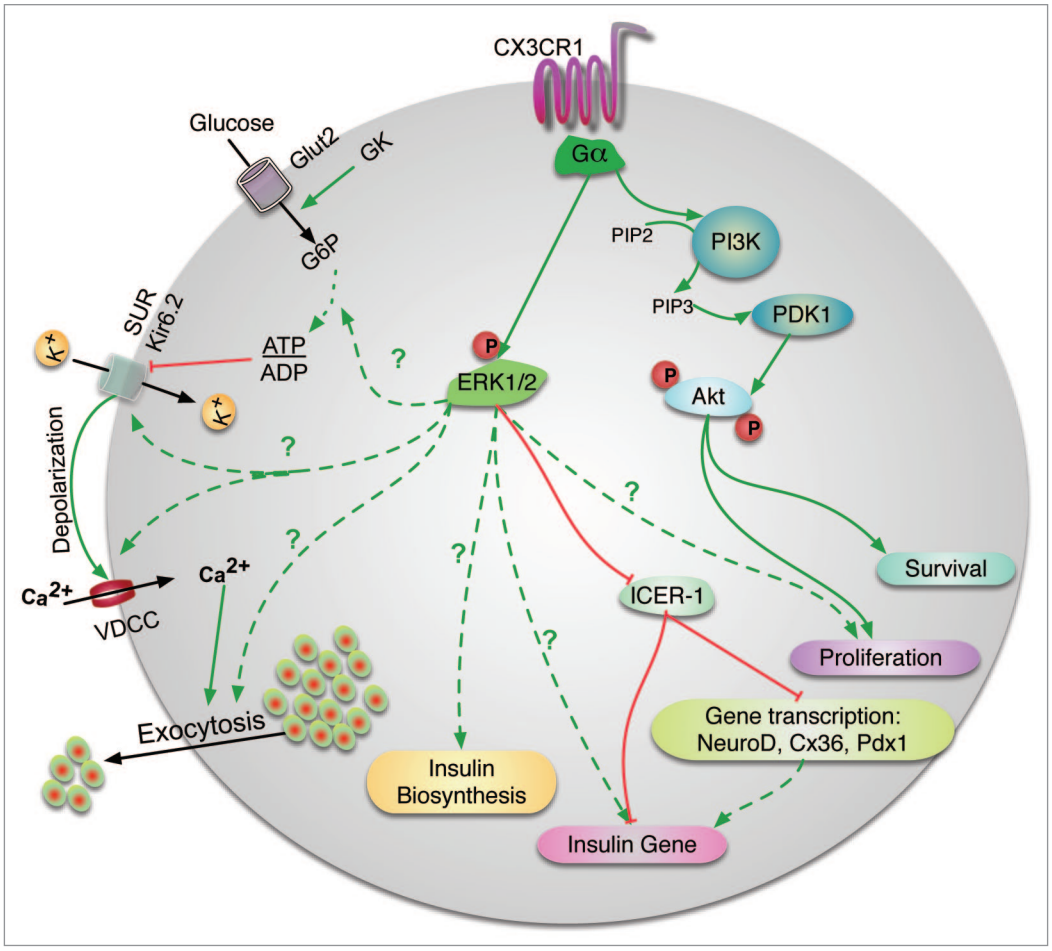

Figure 1. A schematic diagram of downstream signaling in the β cell upon fractalkine receptor ligand binding. Fractalkine, which is also identified as CX3CL1 and neurotactin, is part of the chemokine family of small molecules that induce chemotaxis. In the mouse, fractalkine is the only known ligand of the fractalkine receptor (CX3CR1), a G-protein coupled receptor. Ligand binding leads to an increase in activation of PI3K, MAPK, and Akt signaling, and actin rearrangement. The studies by Lee et al. showed that CX3CR1 deletion and fractalkine therapy results in alterations in survival, gene transcription by modulation of the transcriptional repression ICER1, insulin gene transcription, and insulin content in islets and insulin secretion. It is possible that the alterations in insulin secretion result from modulation of expression of key molecules responsible for this process: glucose transporter (Glut2), glucokinase (GK), other enzymes involved in glycolysis, inward-rectifying potassium channels (Kir6.2), sulphonylurea receptor (SUR), and voltage gated dependent calcium channels (VDCC).

content could be explained by reduced *Insulin* gene transcription as shown by the authors. The alteration of *Insulin* gene transcription could be another consequence of decrease in Erk signaling. Previous studies have shown that Erk1 activation induces *Insulin* gene transcription by phosphorylating Pdx1 and NeuroD and enhancing binding to the E2A3/4 promoter element.[22] It is interesting to speculate that Erk activation following fractalkine receptor signaling may occur through Raf-1 since β-cell Raf-1 knock out animals were found to have an insulin secretory defect which was associated with a decrease in *Ins2* and *NeuroD*.[23] Fractalkine signaling has previously been shown to involve Raf-1 in endothelial cells.[24] Alternatively, it is also possible that the alteration in *Insulin* gene transcription could result from increases in ICER-1 levels, as this negative transcriptional regulator has been shown to repress *Insulin* gene transcription in β cells.[25] Further studies are required to investigate the effect of Fractalkine on *Insulin* gene transcription.

Diabetes and Metabolic Diseases

In addition to the results by Lee and Olefsky et al., there are are multiple reports in the literature implicating fractalkine in the pathogenesis of diabetes. Fractalkine has been found to be elevated in the serum of patients with type 2 diabetes (DM2).[26,27] There have also been associations between fractalkine receptor polymorphisms and obesity[28] as well as DM2 and metabolic syndrome.[27] There was an association found between adult women with the highest quartile of fractalkine levels and elevated fasting insulin levels.[29] Pioglitazone was also shown to decrease serum fractalkine levels in patients with DM2.[30] It is unclear how to integrate these observations with the results of Lee et al. It suggests that, in some circumstances, obesity-induced increases in chemokine production are a compensatory reaction designed to

Table 1. A summary of findings from fractalkine receptor knockout models in a variety of disease states

Effects of Fractalkine Receptor Knock Out		
Deleterious Effects	**Beneficial Effects**	**Variable Effects**
Multiple sclerosis (EAE)[38]	Atherosclerosis[39]	Alzheimer disease[33,40]
Chronic liver disease[31]	Diabetic nephropathy[41]	Macular degeneration[42,43]
Synaptic plasticity[44]	Asthma[45]	
Inflammatory bowel disease[46]	Anxiety behaviors[47]	

sustain insulin secretion by providing feedback from peripheral tissues (e.g., adipose) and islets. It is possible that *CX3CR1* expression is reduced in states of obesity and type 2 diabetes and further studies should explore this possibility. A similar pattern of high serum fractalkine and decreased receptor levels in the target tissue is present in the setting of chronic liver disease.[31] Beyond these few studies there is little mechanistic insight into the role of fractalkine in metabolic disease.

Other Models with Defective Fractalkine/CX3CR1 Signaling

Studies of the fractalkine receptor knockout mice have been complicated by the lack of consistency in the findings using different *Cx3cr1*$^{-/-}$ mice.[32,33] A summary of beneficial and deleterious findings in animal models with a *Cx3cr1*$^{-/-}$ is presented in **Table 1**. In a different set of experiments, Morris et al. showed that *Cx3cr1*$^{-/-}$ mice also in a C57BL/6 background did not prevent the development of obesity-induced insulin resistance or hepatic steatosis. In contrast to the results by Lee et al., these mice show similar glucose metabolism, insulin sensitivity, and hepatic triglyceride content in lean and obese.[15] The cause for the differences in these results is unclear, but it is possible that this could be due to the use of lines generated with different targeting strategies. It also points out some of the potential differences in results between oral and IP glucose tolerance testing—the latter of which is more commonly used by most laboratories. The conflicting results in studies investigating the role of CX3CR1 in other tissues have led one group to propose that knockout animals may attain compensatory mechanisms during development that then lead to decreased release of inflammatory mediators, and that the net effect is not due to the lack of fractalkine signaling.[34] In terms of other studies related to diabetes, a murine model of *Cx3cr1* deficiency in the Akita mouse showed a delay in the development of diabetes but an increase in microglial (retinal macrophage) changes that occur during the development of diabetic retinopathy.[35] The delay in diabetes progression in the Akita model is surprising in light of the detrimental effects on glucose homeostasis observed by Lee et al. Thus, the role of fractalkine signaling can vary based on the specific tissue or disease state, and the presence or absence of fractalkine signaling has an impact on the balance of other local chemokines and inflammatory mediators.

Concluding Remarks

The findings by Lee at al. provide an interesting and novel beneficial effect of fractalkine/CX3CR1 signaling in β-cell function. While the findings have not been demonstrated in other *Cx3cr1*$^{-/-}$ mice, these studies could potentially suggest that fractalkine treatment may make an exciting new therapeutic option to induce insulin secretion and β-cell survival. Fractalkine-based therapeutics are an attractive target based on the exclusivity of the ligand-receptor pairing. This approach must be taken cautiously, however, since some studies have shown that fractalkine receptor mutations and *Cx3cr1*$^{-/-}$ mice have been found to have some degree of protection from coronary artery disease and atherosclerosis.[12] Importantly, in considering its use in diabetes, this pathway has also been shown to be involved in diabetes complications. Fractalkine has been found to play a role in painful neuropathy in the setting of increased serum TNF-α levels in Zucker diabetic fatty rats after the onset of diabetes, thus linking inflammation to the development of this neuropathy.[36] Fractalkine and its receptor have also been found to be upregulated in the kidneys in diabetic animals and are thought to contribute to the progression of diabetic nephropathy.[37] Finally, more studies are required to evaluate the effects of fractalkine therapy on atherosclerosis and diabetes complications before considering this option for therapeutic potential in the setting of diabetes and β-cell dysfunction.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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