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Hematopoietic Tissue Factor–Protease-Activated Receptor 2 Signaling Promotes Hepatic Inflammation and Contributes to Pathways of Gluconeogenesis and Steatosis in Obese Mice

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Failure to inhibit hepatic gluconeogenesis is a major mechanism contributing to fasting hyperglycemia in type 2 diabetes and, along with steatosis, is the hallmark of hepatic insulin resistance. Obesity is associated with chronic inflammation in multiple tissues, and hepatic inflammation is mechanistically linked to both steatosis and hepatic insulin resistance. Here, we delineate a role for coagulation signaling via tissue factor (TF) and proteinase-activated receptor 2 (PAR2) in obesity-mediated hepatic inflammation, steatosis, and gluconeogenesis. In diet-induced obese mice, TF tail signaling independent of PAR2 drives $CD11b^+CD11c^+$ hepatic macrophage recruitment, and TF–PAR2 signaling contributes to the accumulation of hepatic $CD8^+$ T cells. Transcripts of key pathways of gluconeogenesis, lipogenesis, and inflammatory cytokines were reduced in high-fat diet–fed mice that lack the cytoplasmic domain of TF (*F3*) ($TF^{\Delta CT}$) or that are deficient in PAR2 (*F2rl1*), as well as by pharmacological inhibition of TF–PAR2 signaling in diet-induced obese mice. These gluconeogenic, lipogenic, and inflammatory pathway transcripts were similarly reduced in response to genetic ablation or pharmacological inhibition of TF–PAR2 signaling in hematopoietic cells and were mechanistically associated with activation of AMP-activated protein kinase (AMPK). These findings indicate that hematopoietic TF–PAR2 signaling plays a pivotal role in the hepatic inflammatory responses, steatosis, and hepatic insulin resistance that lead to systemic insulin resistance and type 2 diabetes in obesity. *(Am J Pathol 2015, 185: 524–535; http://dx.doi.org/10.1016/j.ajpath.2014.10.008)*

The liver plays a central role in maintaining glucose homeostasis and lipid metabolism, and alterations in liver function underlie the development of cardiovascular and metabolic disease.[1–3] A large proportion of obese patients develops hepatic steatosis, which leads to nonalcoholic fatty liver disease (NAFLD), a disease that ranges from fatty liver to the more severe condition of steatohepatitis, which can, if left unchecked, progress to fibrosis and cirrhosis in up to 25% of patients.[4,5] Alterations of hepatic synthesis of procoagulant and prothrombotic pathway proteins observed in obese subjects with NAFLD further exacerbate the cardiovascular and metabolic risk in this population.[6–9] Another feature of obesity and NAFLD is hepatic inflammation, reflected in the increased production of proinflammatory cytokines and acute-phase proteins and by the activation of pathways regulated by inhibitor of nuclear factor kappa-B kinase subunit beta (IKK-β) and Jun N-terminal kinase (JNK).[10,11] Recent studies suggest a role for hepatic inflammation and immune cells in the regulation of both NAFLD and hepatic insulin resistance. A macrophage population distinct from resident Kupffer cells is recruited to the liver during obesity, and proinflammatory activation of liver macrophages is linked to both insulin resistance and NAFLD.[12,13] However, mechanisms that contribute to the inflammatory state of the obese liver and pathways that drive the recruitment of

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macrophages and other immune cells, including T cells, remain poorly characterized.

Strong clinical evidence suggests that a TF-mediated coagulation pathway is up-regulated in obesity and in metabolic syndrome. Obese subjects have higher plasma concentrations of coagulation factor VII (FVII), increased levels of thrombin and thrombin−antithrombin complexes, and increased circulating monocyte TF procoagulant activity.[14−16] TF initiates the coagulation cascade by serving as the cell surface receptor for FVIIa. In addition to its coagulation functions, TF activates signaling cascades with numerous roles in both normal physiology and disease.[17−19] The TF−FVIIa complex initiates direct TF signaling by activating proteinase-activated receptor 2 (PAR2) and regulates cell migration via a mechanism involving phosphorylation of the TF cytoplasmic domain.[20] Pharmacological and genetic approaches using an antibody (10H10) that specifically blocks TF−PAR2 signaling or mice that lack the cytoplasmic domain of TF or PAR2-deficient mice have provided evidence for TF−PAR2 signaling in cancer progression,[19,21] angiogenesis,[21,22] and inflammation.[23−25] Using such pharmacological and genetic approaches we have established a role for TF−PAR2 signaling in the development of obesity and insulin resistance.[26] We have shown that nonhematopoietic cell TF−VIIa−PAR2 signaling promoted obesity via regulation of overall metabolism and energy expenditure. By contrast, TF−PAR2 signaling in hematopoietic cells attenuated adipose tissue macrophage recruitment and inflammation and led to increased systemic insulin sensitivity. Although these studies suggested that TF-mediated adipose dysfunction may contribute to obesity and systemic insulin resistance, the contribution of hepatic TF signaling to systemic glucose homeostasis and hepatic dysfunction (NAFLD and hepatic insulin resistance) remained to be determined. In the present study, we used genetic and pharmacological tools to unravel a role for TF and PAR2 signaling in hepatic steatosis and insulin resistance. Here, we show that hematopoietic TF−PAR2 signaling blunts AMPK activation, increases hepatic inflammation, and activates pathways of lipogenesis and gluconeogenesis that contribute to hepatic steatosis and insulin resistance, respectively. At the cellular level, TF and PAR2 regulate hepatic recruitment and proinflammatory activation of a population of CD11b$^+$CD11c$^+$ macrophages and CD8$^+$ T cells. Our findings suggest that limiting steatosis and hepatic glucose production by inhibiting TF−PAR2 signaling may provide a novel therapeutic approach for the treatment of type 2 diabetes driven by obesity.

Materials and Methods

Mice

Mice lacking the TF cytoplasmic domain (TF$^{\Delta CT}$), PAR2-deficient mice (*F2rl1*$^{-/-}$; hereafter *Par2*$^{-/-}$), and TF$^{\Delta CT}$/*Par2*$^{-/-}$ mice were all in the C57BL/6J background. Humanized TF knock-in mice (TFKI) in the C57BL/6J background were from Dr. Mark Anderson (Johnson & Johnson Pharmaceutical Research & Development, Spring House, PA). Male mice were fed either a high-fat diet (HFD) (60% kcal from fat) or a low-fat diet (LFD) (10% kcal from fat) (Research Diets, New Brunswick, NJ) beginning at 6 to 8 weeks of age. Bone marrow (BM) chimeras were generated by injecting 5×10^6 to 10×10^6 BM cells at 4 to 6 hours after lethal irradiation of mice. After engraftment under antibiotic prophylaxis for 6 weeks, mice were fed a HFD for 16 to 20 weeks. BM donors typically carried a transgene for green fluorescent protein (GFP) [C57BL/6-Tg(CAG-EGFP)131Osb/LeySopJ; Jackson Laboratory, Bar Harbor, ME], and reconstitution efficiency was determined by analyzing GFP positivity in isolated liver mononuclear cells.

All experiments were approved by the animal ethics committee of the Torrey Pines Institute for Molecular Studies and the Institutional Animal Care and Use Committee of the Scripps Research Institute.

Plasma Insulin, Glucose, and Insulin Tolerance Test

Fasting plasma insulin levels were measured using a mouse ultrasensitive insulin enzyme-linked immunosorbent assay kit (catalog number 80-INSMSU-E01; ALPCO Diagnostics, Salem, NH) according to the manufacturer's instructions. Plasma glucose was measured with a glucometer (Bayer, Leverkusen, Germany). To test for insulin tolerance, mice were injected with insulin (0.75 U/kg; Eli Lilly, Indianapolis, IN), and glucose concentrations were measured in blood samples from tail bleeds at baseline and at 15, 30, 60, 90, and 120 minutes after injection.

Western Blot Analysis

Total protein was extracted from snap-frozen livers in the presence of protease and phosphatase inhibitors, and equal amounts of proteins were separated by 10% SDS PAGE and transferred to nitrocellulose membranes. The membranes were probed with antibodies to AMPKα and phosphorylated AMPKα (p-AMPKα T172) (Cell Signaling Technology, Danvers, MA). Immunoreactive proteins were visualized using an Amersham enhanced chemiluminescence kit (GE Healthcare, Little Chalfont, UK). For analysis of insulin-dependent Akt phosphorylation, mice were injected with 0.75 U/kg insulin via the tail vein 10 minutes before harvesting liver samples for Western blotting using antibodies to Akt and p-Akt (Cell Signaling Technology).

Quantification of Tissue and Plasma Triglyceride

Liver samples (20 to 30 mg) were homogenized for 10 minutes in 1 mL isopropanol using a Bio-Gen Pro200

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