ORIGINAL ARTICLE

A humanized osteopontin mouse model and its application in immunometabolic obesity studies

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Osteopontin (OPN) is a multifunctional protein involved in several inflammatory processes and pathogeneses including obesity-related disorders and cancer. OPN binds to a variety of integrin receptors and CD44 resulting in a proinflammatory stimulus. Therefore, OPN constitutes a novel interesting target to develop new therapeutic strategies, which counteract OPN's proinflammatory properties. We established a humanized *SPP1* (*hSPP1*) mouse model and evaluated its suitability as a model for obesity and insulin resistance. Unchallenged *hSPP1* animals did not significantly differ in body weight and gross behavioral properties compared to wild-type (WT) animals. High-fat diet-challenged *hSPP1* similarly developed obesity and inflammation, whereas insulin resistance was markedly changed. However, OPN expression profile in tissues was significantly altered in *hSPP1* compared to WT depending on the diet. In conclusion, we developed a versatile humanized model to study the action of OPN in vivo and to develop strategies that target human OPN in a variety of pathologies. (Translational Research 2016;■:1–11)

Abbreviations: ■■■ = ■■■

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INTRODUCTION

Osteopontin (OPN) is a multifunctional protein, first identified as a T helper type 1 cytokine, which is mainly expressed in various tissues and cells including macrophages, T-cells, dendritic cells, hepatocytes, smooth muscle cells, endothelial, and epithelial cells.[1-3] OPN exhibits properties not only of a soluble cytokine that acts in an autocrine or paracrine manner on liver cells but also of an extracellular matrix (ECM)-bound protein.[1] OPN plays a prominent role in monocyte migration, adhesion, and differentiation[4-6] as well as in the pathophysiology of chronic inflammatory diseases, such as rheumatoid arthritis,[7] Crohn's disease,[8] cancer,[9] and cardiovascular disease.[10,11] OPN is involved in the pathogenesis of obesity-associated complications, including AT inflammation, insulin resistance, type 2 diabetes, nonalcoholic fatty liver disease, and atherosclerosis.[12-16] In vitro

AT A GLANCE COMMENTARY

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Background

Osteopontin (OPN) is involved in a number of inflammatory processes, including obesity-linked complications, asthma, rheumatoid arthritis as well as cancer progression and metastasis. Proteolytic cleavage of OPN increases OPN's proinflammatory properties and therefore constitutes an interesting target to develop new strategies to counteract OPN's actions. To enable research on human OPN, we generated a humanized *SPP1* (*hSPP1*) mouse and evaluated it as a diet-induced obesity model.

Translational Significance

Our findings propose the *hSPP1* animal model to be applied for a variety of pathologies in which OPN is a key player, including obesity-driven, cardio-metabolic as well as other diseases that can be translated to humans.

studies with adipocytes identified OPN as a modulator of insulin resistance and impaired glucose uptake.[17] Obese mice and humans revealed markedly increased messenger RNA (mRNA) levels for OPN in visceral and subcutaneous fat.[18] Also, plasma OPN concentrations were markedly elevated in obese human subjects compared with lean controls.[19,20] Importantly, high-fat (HF) diet treatment of mice lacking OPN, improved adipose tissue inflammation and insulin sensitivity[12] as well as reduced hyperleptinemia and adipocyte hypertrophy.[21]

OPN acts via integrin binding by different domains. Although the arg-gly-asp (RGD)-containing domain mainly binds integrins $\alpha_v\beta_1$, $\alpha_v\beta_3$, $\alpha_v\beta_5$, and $\alpha_5\beta_1$[22] on the cell surface, the ser-val-val-tyr-glu-leu-arg (SVVYGLR) motif interacts with integrins $\alpha_4\beta_1$, $\alpha_4\beta_7$, and $\alpha_9\beta_1$.[23,24] Importantly, the RGD region is fully conserved between species, whereas the SVVYGLR motif differs between humans (SVVYGLR) and rodents (SLAYGLR).[23] The third integrin-binding domain ELVTDFTDLPAT has also been described to bind $\alpha_4\beta_1$.[25] Overall, human and murine OPN show only 63% amino acid sequence identity (National Center of Biotechnology Information). OPN's actions are affected by protease cleavage. The cryptic region SVVYGLR, which is usually hidden in intact OPN, becomes accessible on thrombin and matrix metalloproteinase cleavage. Interestingly, although enzymatic thrombin and matrix metalloproteinase cleavage produces a proinflammatory N-terminal OPN fragment, the less described C-terminal OPN form may even attenuate inflammatory processes.[26] Moreover, bonding with the hyaluronic acid receptor CD44 leads to the induction of macrophage chemotaxis and the β3-integrin receptors engagement.[27]

To develop therapeutic strategies against OPN actions, small molecules are not a primary option but sequence-specific approaches are needed such as specific antibodies, RNA aptamers, and antisense oligonucleotides or interfering RNAs. Such approaches targeting OPN have been successfully applied on human tissues transplanted into immunodeficient mice, for example, in cancer studies.[28-30] However, human cell transplantation is not feasible for most disease models, particularly inflammatory diseases.

To facilitate in vivo studies of blocking human OPN, we developed a humanized mouse model by replacing the mouse gene with human *SPP1* sequence. Owing to OPN's crucial involvement in a variety of inflammatory pathologies and particularly its association with cardiometabolic complications of obesity, we evaluated the humanized *SPP1* (*hSPP1*) mouse model as a diet-induced obesity (DIO) model. We show the functionality of *hSPP1* mice as a DIO model by generation of obesity-associated insulin resistance and inflammation following HF diet feeding. Hence, *hSPP1* mice are a highly useful model to investigate OPN's role not only to study prevention and treatment of obesity and its complications but also to facilitate preclinical studies on neutralization of human OPN in a large variety of other inflammatory diseases and cancer.

MATERIAL AND METHODS

Animals and diets. Heterozygous C57BL/6-*Spp1*$^{tm2737\ (SPP1)\ Arte}$ (*hSPP1*$^{+/-}$) mice were developed with Taconic (Köln, Germany). In short, the chimeric *SPP1* protein sequence was chosen as depicted in Fig 1, *A*. Accordingly, exon 2 contains the translation initiation codon and the sequence, which encodes the cleavable signal peptide. The mouse genomic sequence from amino acid 21 (valine) in exon 3 up to the termination codon in exon 7 has been replaced by its human counterpart to leave a single N-terminal mouse-specific amino acid in the mature protein (p.Ile19Leu; Fig 1). Positive selection markers, Q5 including neomycin resistance flanked by FRT and puromycin resistance flanked with F3, were inserted into human intron 3 as well as downstream of the mouse 3′ untranslated region, respectively. BAC Q6 clones from the mouse C57BL/6J were used for the targeting vector that was further transfected using

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