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PGRN: a novel therapeutic target and biomarker for insulin resistance and obesity identified by differential proteome analysis

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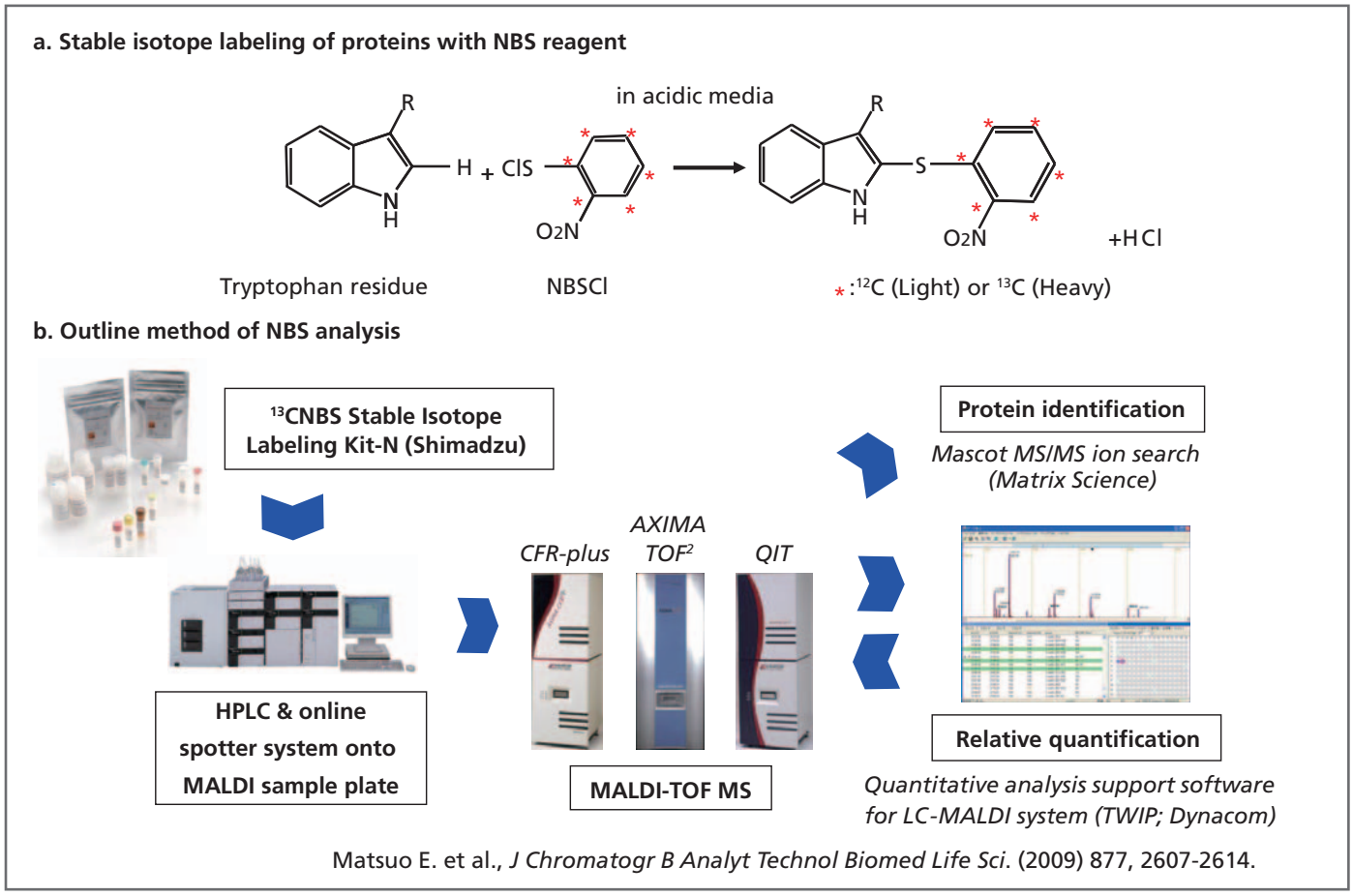
Introduction

Insulin resistance is a characteristic feature of obesity and type 2 diabetes. Adipose tissue is now recognized as not only an energy-storage tissue, but also an endocrine tissue that secretes a variety of bioactive substances (adipokines). Defects in adipokine secretion accompanying adipose tissue dysfunction contribute to the pathophysiology of insulin resistance and obesity. The relationship between inflammatory process and insulin resistance has recently drawn considerable attention. For example, TNF-alpha, a proinflammatory cytokine, has been shown to contribute to the development of insulin resistance. On the other hand, glucocorticoids, which are known to have an anti-inflammatory action, also induce insulin resistance in human and animals. Dexamethasone, a glucocorticoid, has been reported to impair insulin signaling and insulin-stimulated glucose uptake in adipose tissue, liver, and skeletal muscle. Since TNF-alpha and dexamethasone both induce insulin resistance despite their opposite inflammatory properties, we reasoned that there might be a common key mediator responsible for the cellular basis of insulin resistance induced by TNF-alpha and dexamethasone. In the present study, we searched for a novel adipokine(s) that play a key role in developing insulin resistance using 3T3-L1 adipocytes treated with TNF-alpha or dexamethasone. For this purpose, we utilized a method of differential proteome analysis based on stable isotope labeling of proteins with chemical reagent 2-nitrobenzenesulfenyl chloride (NBSCl) incorporating six ^{13}C ($^{13}C_6$) or six ^{12}C ($^{13}C_0$) in the tryptophan residues (Fig.1a). The aim of this study is to search for a novel adipokine(s) that play a key role in developing insulin resistance.



Matsuo E. et al., *J Chromatogr B Analyt Technol Biomed Life Sci.* (2009) 877, 2607-2614.

Fig. 1 NBS Biomarker Discovery System

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Methods

To identify proteins associated with insulin resistance in adipocytes *in vitro*, differential proteome analysis using the NBS method was performed in 3T3-L1 adipocytes in which insulin resistance was induced by TNF-alpha or dexamethasone (Fig. 2a). The relative quantification and the identification of differentially expressed proteins were performed using LC-MALDI-TOF MS (Fig. 1b and 2a).

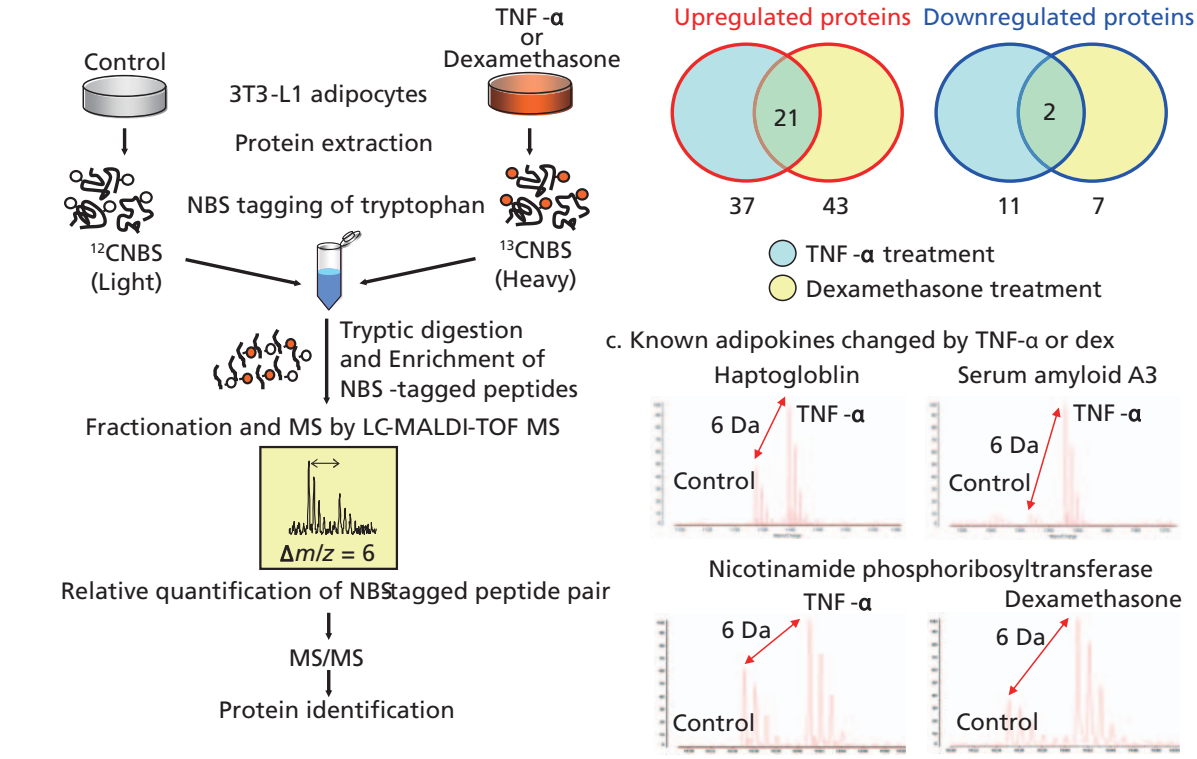

Fig. 2 Differential proteome analysis of insulin resistance associated proteins in 3T3-L1 adipocytes

Results

We found that 37 and 43 proteins were upregulated by TNF-alpha treatment and dexamethasone treatment, respectively, among which 21 proteins are common in the two treatments (Fig. 2b). We also found that 11 and 7 proteins were downregulated by TNF-alpha treatment and dexamethasone treatment, respectively, among which 2 proteins are common in these treatments (Fig. 2b). Identification of haptoglobin, serum amyloid A-3 (SAA3) protein precursor and nicotinamide phosphoribosyltransferase (Fig. 2c), all of which are known as the adipokines to be upregulated by such treatment, confirmed the validity of the method. After excluding known adipokines among the 23 proteins identified, we confirmed the results of differential proteome analysis on 8 proteins by immunoblot analysis using antibodies currently available (data not shown). We finally selected progranulin (PGRN) because it is the only protein with both secretory and proinflammatory properties and further analyzed the protein functions.

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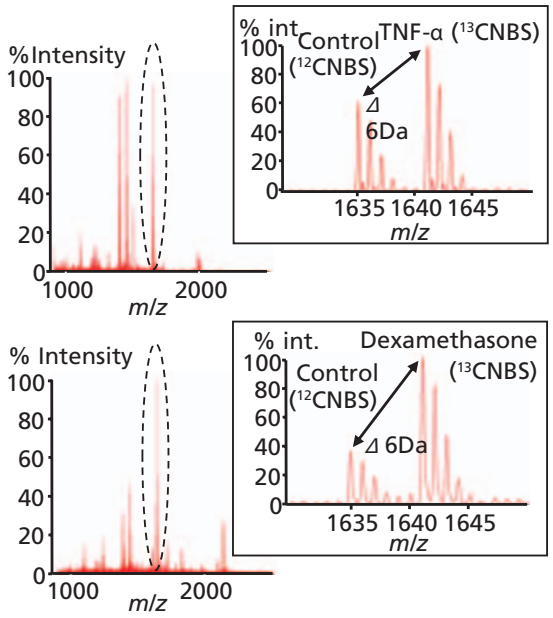
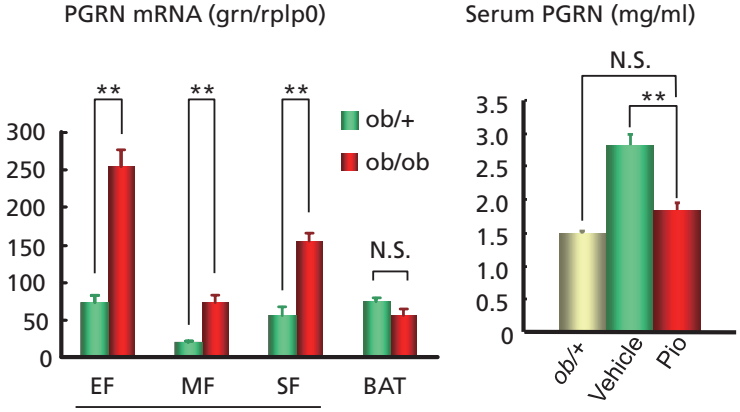
PGRN was upregulated in both TNF-alpha-treated (1.66-fold vs. control) and dexamethasone-treated (3.01-fold vs. control) adipocytes (Fig. 3a). PGRN in blood and adipose tissues was markedly increased in obese mouse models and was normalized with treatment of pioglitazone, an insulin-sensitizing agent. PGRN induced insulin resistance *in vivo* (Fig. 3c). Ablation of PGRN prevented mice from high-fat diet (HFD)-induced insulin resistance (data not shown), adipocyte hypertrophy (Fig. 3d, HE staining), and obesity (Fig. 3d).

a. PGRN levels in 3T3-L1 adipocytes



PGRN (aa297-209): R.LNTGAW*GCCPFAK.A

b. Tissues and serum levels of PGRN *in vivo*

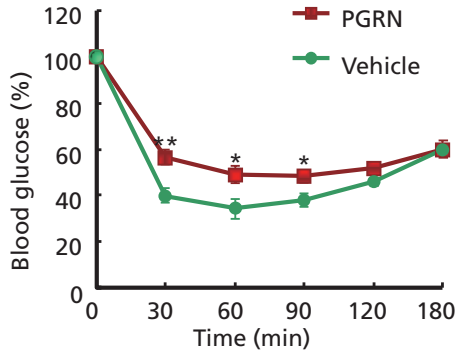


White adipose tissue

EF: Epididymal fat
MF: Mesenteric fat
SF: Subcutaneous fat

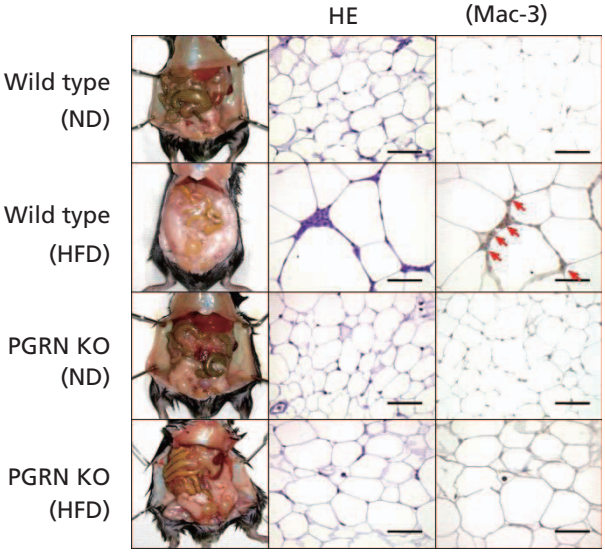
Vehicle (0.25% CMC)
Pioglitazone (30 mg/kg) for 7days

c. Insulin tolerance test in mice chronically administered with PGRN



PGRN: recombinant protein, Vehicle: PBS
ip for 2 weeks (20 μg/day)
P -value * <0.05, ** <0.01.

d. Phenotypic analysis of PGRN knockout mice Macrophage



ND: Normal diet
HFD: High-fat diet

Fig. 3 Identification and functional analysis of novel adipokine

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Conclusions

PGRN is a key adipokine that mediates HFD-induced insulin resistance and obesity through production of IL-6 in adipose tissue, and may be a promising therapeutic target for obesity. Differential proteome analysis based on the NBS method described here should be useful for identifying proteins involved in insulin resistance in various tissues.

References

Matsubara T. et al., PGRN is a key adipokine mediating high fat diet-induced insulin resistance and obesity through IL-6 in adipose tissue. Cell Metab. (2012) 15, 38-50.

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