LECT2 functions as a hepatokine that links obesity to skeletal muscle insulin resistance.

【総説】

第13回　高安賞優秀論文賞受賞

論文　「LECT2 functions as a hepatokine that links obesity to skeletal muscle insulin resistance.」

Diabetes

2014年5月　掲載

Lan F, Misu H, Chikamoto K, Takayama H, Kikuchi A, Mohri K, Takata N, Hayashi H, Matsuzawa-Nagata N, Takeshita Y, Noda H, Matsumoto Y, Ota T, Nagano T, Nakagen M, Miyamoto KI, Takatsuki K, Seo T, Iwayama K, Tokuyama K, Matsugo S, Tang H, Saito Y, Yamagoe S, Kaneko S, Takamura T.

肥満関連ヘパトカインLECT2は骨格筋インスリン抵抗性を発症させる

蘭　　菲（らん　ふぇい）

Background

Insulin resistance is a characteristic feature of people with type 2 diabetes[1] and plays a major role in the development of various diseases such as cardiovascular diseases and nonalcoholic steatohepatitis[2]. However, the molecular mechanisms underlying insulin resistance are now known to be influenced by the abnormal secretion of tissue-derived factors, such as adipokines, myokines, and hepatokines[3]. Leukocyte cell-derived chemotaxin 2 (LECT2) is a protein preferentially produced by human adult and fetal liver cells and is secreted into the blood stream[4]. To date, however, the role of LECT2 in the development of obesity and insulin resistance induced by over-nutrition has not yet been established. In the present study, we have identified LECT2 as a hepatokine whose expression levels were positively correlated with the severity of obesity in humans. Blood levels of LECT2 were also elevated in animal models with obesity. *Lect2*-deficient mice showed an increase of insulin signaling in skeletal muscle. Conversely, treatment with recombinant LECT2 protein impaired insulin signaling in C2C12 myotubes. Our data demonstrate that LECT2 functions as a hepatokine that links obesity to insulin resistance in the skeletal muscle.

Results

To characterize the role of LECT2 in humans, we measured serum LECT2 levels in participants who visited the hospital for a complete physical examination. We found a significant positive correlation between serum LECT2 levels and BMI, waist circumference, homeostasis model assessment of insulin resistance (HOMA-R), hemoglobin A1c (HbA1c) and systolic blood pressure. These results indicate that serum levels of LECT2 are positively associated with both adiposity and the severity of insulin resistance in humans.

To explore the regulation of LECT2 in liver, we fed C57BL6J mice with a high fat diet (HFD) for 8 weeks, and found serum levels of LECT2 showed a sustained increase since a week after the beginning of HFD. Importantly, the livers from mice fed a HFD for 8 weeks showed a decrease of phosphorylation of adenosine monophosphate-activated protein kinase (AMPK). LECT2 levels were elevated in blood obtained from fed mice, compared with samples from the fasting mice. Moreover, AMPK phosphorylation decreased in the livers from the mice that had been fed. Since LECT2 expression was inversely correlated with AMPK phosphorylation in the liver, we hypothesized that AMPK negatively regulates LECT2 production in the hepatocytes. Thus, C57BL6J mice were loaded onto a running treadmill for a total of 3 h. Exercise decreased levels of gene expression and protein for LECT2 in the liver and significantly reduced serum levels of LECT2. Then we transfected H4IIEC hepatocytes with adenoviruses either encoding constitutively active (CA-) or dominant-negative (DN-) AMPK. Transfection with CA-AMPK significantly decreased mRNA levels for *Lect2* in H4IIEC hepatocytes, In contrast, transfection with DN-AMPK increased *Lect2* gene expression. These results indicate that AMPK negatively regulates LECT2 production in the hepatocytes.

Next, we examined the role of LECT2 in the development of insulin resistance in systemic knockout mice of LECT2. Body weight, food intake, and resting heat production were unaffected by *Lect2* knockout. However, the treadmill running challenge revealed that physical-exercise-assessed muscle endurance was significantly higher in $Lect2^{-/-}$ mice. A glucose or insulin loading test revealed that $Lect2^{-/-}$ mice

showed lower blood glucose levels after glucose or insulin injection. *Lect2*$^{-/-}$ mice exhibited an increase in insulin-stimulated Akt phosphorylation in skeletal muscle, but not in the liver or adipose tissue. Consistent with the results of insulin signaling, hyperinsulinemic-euglycemic clamp studies showed that the glucose infusion rate and peripheral glucose disposal were increased, whereas endogenous glucose production was unaffected by *Lect2* deletion. These results indicate that a *Lect2* deletion increases insulin sensitivity in the skeletal muscle in mice.

To further elucidate the role of LECT2 in the development of obesity-associated insulin resistance, we fed *Lect2*-deficient mice a HFD. HFD-induced body weight gain was smaller in *Lect2*-deficient mice compared with wild-type animals. 11 weeks later after HDF feeding, serum levels of insulin and blood levels of glucose decreased in these knockout mice. A glucose or insulin loading test revealed that *Lect2*-knockout mice showed lower blood glucose levels after glucose or insulin injection. Western blotting revealed that insulin-stimulated Akt phosphorylation increased in skeletal muscle of these knockout animals. In contrast, c-Jun-N-terminal kinase (JNK) phosphorylation significantly decreased in skeletal muscle of *Lect2*-deficient mice. These results indicate that *Lect2* deletion reduces muscle insulin resistance in dietary obese mice.

To examine the effect of LECT2 on insulin signaling *in vitro*, we transfected C2C12 myocytes with a plasmid expression vector encoding mouse LECT2. The cells transfected with the LECT2 vector showed a decrease in insulin-stimulated Akt phosphorylation and an increase in basal JNK phosphorylation. To further confirm the acute action of LECT2 on insulin signaling, we treated C2C12 myotubes with recombinant LECT2 protein at nearly physiological concentrations. Treatment with 400 ng/ml of LECT2 protein for 3 h decreased insulin-stimulated Akt phosphorylation. In addition, treatment with LECT2 protein for 30-60 min increased JNK phosphorylation transiently in C2C12 myotubes. LECT2-induced JNK phosphorylation was observed to occur in a concentration-dependent manner. To determine whether JNK pathway mediates LECT2-induced insulin resistance, we transfected C2C12 myoblasts with siRNAs specific for JNK1 and JNK2. Because knockdown of JNK is known to alter the myotube-differentiation in C2C12 myotube, we used undifferentiated C2C12 myoblasts to purely assess the action of LECT2 on insulin signal transduction in the following experiments. Double knockdown of JNK1 and JNK2 rescued the cells from the inhibitory effects of LECT2 on insulin signaling. These *in vitro* experiments indicate that, at nearly physiological concentrations, LECT2 impairs insulin signal transduction by activating JNK in C2C12 myocytes.

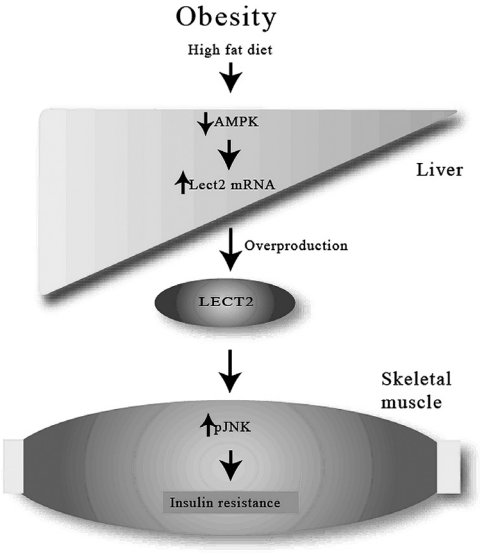


Fig 1. The hepatokine LECT2 links obesity to insulin resistance in the skeletal muscle. (Diabetes. 2014 May;63 p1162)

Conclusion

We demonstrate that leukocyte cell-derived chemotaxin 2, as an energy-sensing hepatokine, is a link between obesity and skeletal muscle insulin resistance. Our researches reveals that high fat diet or over nutrition up-regulates LECT2 production by decreasing AMPK phosphorylation in the liver on obese conditions. As a result, the overproduction of Lect2 contributes to the development of muscle insulin resistance by increasing JNK phosphorylation (Fig 1). These results demonstrate a previously-unrecognized role of LECT2 in glucose metabolism, and suggest that Lect2 may be a novel therapeutic target for obesity-associated insulin resistance.

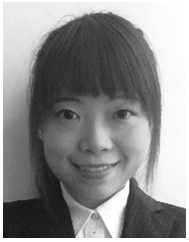
Reference

1) Saltiel AR, Kahn CR. Insulin signalling and the regulation of glucose and lipid metabolism. Nature 2001; 414: 799-806

2) Takamura T, Misu H, *et al.* Fatty liver as a consequence and cause of insulin resistance: lessons from type 2 diabetic liver. Endocr J 2012; 59: 745-763

3) Misu H, Takamura T, *et al.* A liver-derived secretory protein, selenoprotein P, causes insulin resistance. Cell Metab 2010; 12: 483-495

4) Yamagoe S, Mizuno S, Suzuki K. Molecular cloning of human and bovine LECT2 having a neutrophil chemotactic activity and its specific expression in the liver. Biochim Biophys Acta 1998; 1396: 105-113



Profile

2003-2010 West China School of Medicine, Sichuan University, P.R.China. MD.

2010-2014 Kanazawa University Graduate School of Medical Science, Japan. Ph.D.