Neuregulin-4 is an angiogenic factor that is critically involved in the maintenance of adipose tissue vasculature

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(Degree)
博士（医学）

(Date of Degree)
2018-09-25

(Resource Type)
doctoral thesis

(Report Number)
甲第7282号

(URL)
https://hdl.handle.net/20.500.14094/D1007282

学位論文の内容要旨

Neuregulin-4 is an angiogenic factor that is critically involved in the maintenance of adipose tissue vasculature

Neuregulin-4は脂肪組織の血管維持に重要な役割を果たす血管新生因子である

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SUMMARY

Obesity is a pandemic and it certainly has become a global health issue. Obesity causes systemic metabolic disorders by inducing insulin resistance, and consequently increases the morbidity and mortality of atherosclerotic diseases such as stroke and myocardial infarction . Chronic inflammation in WAT plays a fundamental role in obesity-associated adipocyte dysfunction, and adipose tissue hypoxia is an emerging factor that triggers and/or exacerbates inflammatory mechanisms in WAT. WAT contains well-developed vascular networks; however, the rapid expansion of adipocytes during obesity causes imbalanced angiogenesis, leading to a reduction in blood vessel density and consequent hypoxia in AT .

Brown adipose tissue (BAT) is one of the most vascularized tissues throughout the body, and it fulfills the demand for oxygen and nutrient supplies for heat generation. Blood perfusion of BAT determines the thermogenic capacity, whereas thermogenesis in BAT makes a great impact on the whole-body energy expenditure. Accordingly, angiogenesis in BAT is integrally involved in metabolic rate and adiposity. Obesity also causes BAT blood vessel rarefaction, leading to a decline in thermogenic capacity and energy expenditure, which further exacerbates obesity. Therefore, AT angiogenesis is closely associated with adipocyte functions, and is implicated in systemic energy metabolism and glucose homeostasis. However, it remains unclear whether vascular rarefaction in AT is simply secondary to excessive adipocyte hypertrophy, or if AT angiogenesis is negatively regulated (or actively inhibited) during the progression of obesity. We searched for genes highly 4 expressed in healthy WAT and are down-regulated in obesity; accordingly, we identified neuregulin-4 (Nrg4) as one of such growth factors. It has been reported that Nrg4 expression was significantly enhanced by cold exposure in WAT, and that Nrg4 was involved in systemic metabolic homeostasis by modulating lipogenic gene expression in liver. However, its role in AT angiogenesis remained to be unknown. Here, we revealed that Nrg4 is an angiogeic growth factor, and is critically involved in the maintenance of AT vascular networks.

To identify unique pathway(s) in AT angiogenesis, which is impaired in obesity, we performed microarray analysis using RNAs prepared from WAT of lean or obese mice. We searched for growth factors that are highly expressed in healthy WAT and down-regulated in WAT of obese mice, and identified Nrg4, the newest member of neuregulins that belong to the EGF family of extracellular ligands. Neuregulins are

membrane proteins, and their extracellular domain that contains a characteristic EGF-like domain is released through shedding and functions as a growth factor through activating ErbB receptors. Among neuregulin family members, Nrg4 shows unique structural features such as a small size and short intracellular domain. Of note, its tissue distribution was strikingly different from that of other family members. Nrg4 was highly and preferentially expressed in AT, whereas other members were predominantly expressed in brain.

In consistent with previous reports, Nrg4 is primarily expressed in mature adipocytes, and its expression in WAT and BAT was reduced by diet-induced obesity. Treatment with inflammatory cytokine and endoplasmic reticulum (ER)-stress inducer significantly reduced Nrg4 expression in 3T3-L1 adipocytes, suggesting that these stresses may cause the decline in Nrg4 expression in WAT during obesity.

We then examined the expression of Nrg4 in various types of cells, and confirmed its high and preferential expression in 3T3-L1 mature adipocytes, while its receptor ErbB4 is highly expressed in endothelial cells. Immunohistochemistry for ErbB4 in WAT and BAT showed that most of endothelial cells in AT vasculature appeared to express ErbB4. These results suggest that adipocyte-derived Nrg4 might activate endothelial cells in AT vasculature.

Functionally, recombinant Nrg4 significantly enhanced proliferation and tube-formation of endothelial cells, whereas it reduced endothelial apoptosis. Moreover, Nrg4 significantly enhanced in vivo angiogenesis in Matrigel-plugs that were subcutaneously implanted in mice. These data indicate that Nrg4 is a novel angiogenic factor preferentially produced by adipocytes.

To explore the role of Nrg4 in AT angiogenesis in vivo, we generated mice in which Nrg4 was genetically deleted. Loss of Nrg4 caused reduction in blood vessels both in WAT and BAT. Interestingly, Nrg4−/− mice showed increased body weight and adiposity even under normal dietary condition, while their food intake and muscle mass were similar compared with WT mice.

Expression of some thermogenic genes decreased in BAT, and oxygen consumption was reduced without decline in activity in Nrg4−/− mice compared with those in WT mice. These findings suggest that loss of Nrg4 caused reduction in BAT blood vessels and impaired thermogenic capacity, which resulted inreduced energy expenditure and consequent overweight. Of note, Nrg4−/− mice showed reduced insulin sensitivity and impaired glucose tolerance compared with WT mice even while consuming normal chow. Expression of several inflammatory genes showed tendency toward increase, and the adiponectin expression was considerably reduced in WAT of Nrg4−/− mice when compared with those in WT mice. These data collectively indicate that Nrg4 is essential to maintain AT vasculature, failure of which causes adipocyte dysfunction, leading to impaired metabolic health even under normal nutritional condition.

To further validate a role of adipocyte-derived Nrg4 in AT angiogenesis, we generated mice in which Nrg4 was specifically deleted in brown adipocytes (Nrg4-bKO). We confirmed the specific silencing of Nrg4 in BAT in Nrg4-bKO mice. Of note, loss of Nrg4 in brown adipocytes reduced blood vessels in BAT but not in WAT. Expression of some genes associated with thermogenesis was reduced in BAT of Nrg4-bKO mice comparing with that in control Nrg4-flox mice, though the gene expression pattern was different from that in Nrg4-null-KO mice. In contrast to Nrg4-null-KO mice, adiponectin expression in WAT was similar between Nrg4-flox and Nrg4-bKO mice. In a way similar to Nrg4-null-KO mice, Nrg4-bKO mice showed increased body weight and adiposity with no changes in food intake under normal nutritional condition. Also, Nrg4-bKO mice showed reduced insulin sensitivity and impaired glucose tolerance in a way similar to Nrg4-null-KO mice.

These findings collectively indicate that adipocyte-derived Nrg4 plays a critical role in maintaining AT vasculature and its metabolic functions in a paracrine manner

In the current study, we revealed a previously undescribed mechanism in the regulation of AT angiogenesis via Nrg4. Obesity often accompany the AT vascular rarefaction, and obesity and its-related metabolic disorders could be prevented by enhancing AT angiogenesis. In this study, we identified a novel angiogenic role of Nrg4, and found that adipocyte-derived Nrg4 is essential to maintain AT vasculature and adipocyte metabolic functions under normal nutritional condition. Nrg4 is highly expressed in healthy adipocytes and its expression was down-regulated in obesity; therefore, Nrg4-mediated AT angiogenesis is probably inhibited in obesity, which could play a causative role in obesity-associated AT vascular rarefaction. Given that Nrg4-bKO mice showed metabolic phenotype similar to Nrg4-null-KO mice, BAT dysfunction due to reduced blood vessels dominantly contributes to the impaired metabolic health induced by loss of Nrg4 under normal nutritional condition. However, reduction of blood vessels and adiponectin expression in WAT, which was detected in Nrg4-null-KO mice, was not observed in Nrg4-bKO mice, suggesting a role of WAT-

derived Nrg4 in adipocyte functions and metabolic control as well. During our work to characterize Nrg4 angiogenic functions, several studies regarding Nrg4 were reported. One study showed that Nrg4 is a BAT-enriched gene and its expression was significantly enhanced by cold exposure in WAT. In this study, potential role of Nrg4 in neurite outgrowth in PC12 pheochromocytoma cells was also revealed. Other study reported that Nrg4 is a brown adipocytes-derived endocrine factor that modulates the expression of lipogenic genes in liver and consequently preserves metabolic homeostasis under obese condition. AT angiogenesis was not analyzed, and the metabolic effects of Nrg4-deletion in mice under normal dietary condition was not characterized in detail in the previous works. Furthermore, we generated BAT-specific Nrg4-deficient mice for the first time, and revealed a critical role of adipocyte-derived Nrg4 in the maintenance of AT vasculature and its metabolic functions in a paracrine manner. Many growth factors have various functions depending on target cells; therefore, Nrg4 could be a multifunctional growth factor targeting neuronal cells, hepatocytes, and endothelial cells.

Our findings emphasize the important and active roles of AT angiogenesis in the maintenance of AT metabolic functions, and thus support the rationale for AT angiogenesis as a potential therapeutic target; however, AT-targeting therapy is certainly required because angiogenesis plays an important role in various biological processes including tumor progression.

神戸大学大学院医学(系)研究科（博士課程）

論文審査の結果の要旨

受付番号	甲 第2804号	氏名	DHITE BAYU NUGROHO
論文題目 Title of Dissertation	Neuregulin-4は脂肪組織の血管維持に重要な役割を果たす血管新生因子である Neuregulin-4 is an angiogenic factor that is critically involved in the maintenance of adipose tissue vasculature		
審査委員 Examiner	主査 Chief Examiner 小川渉 副査 Vice-examiner 古屋敷智之 副査 Vice-examiner 鈴木聡		

（要旨は１，０００字～２，０００字程度）

In this study, the authors searched for growth factors whose expression in AT is down-regulated in obesity. They have performed microarray analysis using RNAs prepared from WAT of lean or obese mice to identify unique pathway(s) in AT angiogenesis, which is impaired in obesity. They searched for growth factors that are highly expressed in healthy WAT and down-regulated in WAT of obese mice, and identified Nrg4, the newest member of neuregulins that belong to the EGF family of extracellular ligands. Among neuregulin family members, Nrg4 shows unique structural features such as a small size and short intracellular domain. Of note, its tissue distribution was strikingly different from that of other family members. Nrg4 was highly and preferentially expressed in AT, whereas other members were predominantly expressed in brain. Nrg4 is primarily expressed in mature adipocytes, and its expression in WAT and BAT was reduced by diet-induced obesity. Nrg4 is primarily expressed in mature adipocytes, and its expression in WAT and BAT was reduced by diet-induced obesity. Treatment with inflammatory cytokine and endoplasmic reticulum (ER)-stress inducer significantly reduced Nrg4 expression in 3T3-L1 adipocytes, suggesting that these stresses may cause the decline in Nrg4 expression in WAT during obesity. They then examined the expression of Nrg4 in various types of cells, and confirmed its high and preferential expression in 3T3-L1 mature adipocytes, while its receptor ErbB4 is highly expressed in endothelial cells. Immunohistochemistry for ErbB4 in WAT and BAT showed that most of endothelial cells in AT vasculature appeared to express ErbB4.

To explore the role of Nrg4 in AT angiogenesis in vivo, the authors generated mice in which Nrg4 was genetically deleted. Nrg4−/− mice showed increased body weight and adiposity even under normal dietary condition, while their food intake and muscle mass were similar compared with WT mice. Expression of some thermogenic genes decreased in BAT, and oxygen consumption was reduced without decline in activity in Nrg4−/− mice compared with those in WT mice, suggesting that loss of Nrg4 caused reduction in BAT blood vessels and impaired thermogenic capacity. Nrg4−/− mice showed reduced insulin sensitivity and impaired glucose tolerance.

To further validate a role of adipocyte-derived Nrg4 in AT angiogenesis, the authors generated mice in which Nrg4 was specifically deleted in brown adipocytes (Nrg4-bKO). Loss of Nrg4 in brown adipocytes reduced blood vessels in BAT but not in WAT. Expression of some genes associated with thermogenesis was reduced in BAT of Nrg4-bKO mice comparing with that in control Nrg4-flox mice, though the gene expression pattern was different from that in Nrg4-null-KO mice. In contrast to Nrg4-null-KO mice, adiponectin expression in WAT was similar between Nrg4-flox and Nrg4-bKO mice. In a way similar to Nrg4-null-KO mice, Nrg4-bKO mice showed increased body weight and adiposity with no changes in food intake under normal nutritional condition. Also, Nrg4-bKO mice showed reduced insulin sensitivity and impaired glucose tolerance in a way similar to Nrg4-null-KO mice. These findings collectively indicate that adipocyte-derived Nrg4 plays a critical role in maintaining AT vasculature and its metabolic functions in a paracrine manner.

The candidate, having completed studies on Neuregulin-4 in the regulation of the vasculature and the thermogenic function in brown adipose tissue, and having advanced the filed of knowledge in the area of the pathogenesis and the treatment of obesity, is hereby recognized as having qualified for the degree of Ph. D (Medicine).