Role of Adiponectin in Preventing Vascular Stenosis

THE MISSING LINK OF ADIPO-VASCULAR AXIS

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Obesity is more linked to vascular disease, including atherosclerosis and restenotic change, after balloon angioplasty. The precise mechanism linking obesity and vascular disease is still unclear. Previously we have demonstrated that the plasma levels of adiponectin, an adipose-derived hormone, decreases in obese subjects, and that hypoadiponectinemia is associated to ischemic heart disease. In current the study, we investigated the in vivo role of adiponectin on the neointimal thickening after artery injury using adiponectin-deficient mice and adiponectin-producing adenovirus. Adiponectin-deficient mice showed severe neointimal thickening and increased proliferation of vascular smooth muscle cells in mechanically injured arteries. Adenovirus-mediated supplementation of adiponectin attenuated neointimal proliferation. In cultured smooth muscle cells, adiponectin attenuated DNA synthesis induced by growth factors including platelet-derived growth factor, heparin-binding epidermal growth factor (EGF)-like growth factor (HB-EGF), basic fibroblast growth factor, and EGF and cell proliferation and migration induced by HB-EGF. In cultured endothelial cells, adiponectin attenuated HB-EGF expression stimulated by tumor necrosis factor α. The current study suggests an adipo-vascular axis, a direct link between fat and artery. A therapeutic strategy to increase plasma adiponectin should be useful in preventing vascular restenosis after angioplasty.

Obesity is a common risk for insulin resistance and cardiovascular diseases (1, 2). However, the molecular mechanism of the relationship between obesity and vascular diseases remains unclear. Adipocytes produce and secrete a variety of biologically active molecules, conceptualized as adipocytokines, including tumor necrosis factor (TNF) α, 1 leptin, resistin, plasminogen activator inhibitor-1 and heparin-binding epidermal growth factor (EGF)-like growth factor (HB-EGF) (3–7). Several lines of evidences suggest that dysregulated production of adipocytokines participates in the development of metabolic and vascular diseases related to obesity (3–7).

Adiponectin is an adipocyte-derived factor that was identified by our group in human adipose tissues (8). Acrp30 or AdipoQ, independently cloned by two groups, is the mouse counterpart of adiponectin (9, 10). Adiponectin mRNA is expressed exclusively in adipose tissues. Adiponectin is composed of two structurally distinct domains: C-terminal collagen-like fibrous domain and complement C1q-like globular domain. Interestingly, low plasma concentrations of adiponectin are found in obese subjects (11) and patients with coronary artery disease (12). Furthermore, the incidence of cardiovascular death is higher in renal failure patients with low plasma adiponectin compared with those with higher plasma adiponectin levels (13). We have also reported that adiponectin infiltrates rapidly into the subendothelial space of the vascular wall when the endothelial barrier of the arterial wall is injured by balloon angioplasty (14). In tissue cultures, adiponectin attenuates monocyte attachment to endothelial cells by reducing the expression of adhesion molecules on endothelial cells (12, 15). Adiponectin also suppresses lipid accumulation in monocyte-derived macrophages through the suppression of macrophage scavenger receptor expression (16). These in vitro data suggested the anti-atherogenic properties of adiponectin, and hence hypoadiponectinemia might be associated with a higher incidence of vascular diseases in obese subjects.

In the present study, we investigated the role of adiponectin on the vascular wall in vivo using adiponectin knockout (KO) mice and adiponectin-producing adenovirus (17). Our results demonstrate that adiponectin deficiency aggravates neointimal thickening, and adiponectin supplement attenuates neointimal thickening in mechanically injured arteries, presumably through the suppressive effect of adiponectin on the proliferation and migration of vascular smooth muscle cells. Here we show the first in vivo evidence that adiponectin is a fat-derived hormone directly bridging the adipose-vascular axis.

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The abbreviations used are: TNFα, tumor necrosis factor α; EGF, epidermal growth factor; HB-EGF, heparin-binding EGF-like growth factor; KO, knockout; WT, wild-type; APN, adiponectin; Ad-APN, adenovirus-adiponectin; Ad-fgal, adenovirus-f-galactosidase; HASMC, human aortic smooth muscle cell; HAE, human aortic endothelial cell; PDGF, platelet-derived growth factor; FGF, fibroblast growth factor; I/M ratio, intimal/medial area ratio; VSMC, vascular smooth muscle cell.
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Phenotypic comparison in wild-type and adiponectin knockout mice

| Phenotypic Comparison | Wild-type | Knockout |
|-----------------------|-----------|----------|
| **Body weight (g)**   | 30.0 ± 2.2| 30.9 ± 0.9|
| **Epididymal fat (g)**| 0.65 ± 0.07| 0.67 ± 0.08|
| **Liver (g)**          | 1.74 ± 0.16| 1.74 ± 0.84|
| **Heart (g)**          | 0.59 ± 0.01| 0.59 ± 0.01|
| **Plasma glucose (mg/dl)**| 202.8 ± 4.6| 250.7 ± 18.3|
| **Plasma insulin (ng/ml)**| 0.20 ± 0.04| 0.32 ± 0.14|
| **Plasma triglyceride (mg/dl)**| 37.1 ± 6.5| 48.4 ± 7.0|
| **Plasma cholesterol (mg/dl)**| ND| ND|
| **FFA (mm)**           | 0.92 ± 0.22| 0.76 ± 0.18|

**RESULTS**

**Basal Profile of Adiponectin Knockout Mice**—Adipose mRNA and plasma protein of adiponectin were deficient in KO mice studied in the current analysis (data not shown). Table I describes the phenotypic comparison in WT and adiponectin KO mice under non-fasted and 12-h-fasted conditions. No significant differences were observed in the weights of body and various tissues including epididymal white fat, brown fat, liver, gastrocnemius muscle, and heart. Plasma concentration of glucose, insulin, cholesterol, triglyceride, and free fatty acid were not altered significantly in the adiponectin KO mice.
Adiponectin Deficiency Increases Neointimal Thickening in Injured Arteries—In the present study, we denuded the vascular endothelium of the femoral artery as we described previously (19, 20) and compared the neointimal thickening of the arteries at 3 weeks after WT and KO mice. Hematoxylin-eosin staining (Fig. 1A, upper panel) demonstrated that the neointimal hyperplasia in the injured artery was worse in KO mice than in WT mice. Immunohistochemical staining revealed that the neointima in both WT and KO mice was composed of α-smooth muscle actin--positive smooth muscle cells (Fig. 1A, lower panel). We also quantitatively measured the intimal and medial area by computerized morphometry (Fig. 1B). The I/M ratio (the ratio of intimal area/medial area) was significantly greater in KO mice compared with WT mice (p < 0.01). Notably, two of seven injured femoral arteries were occluded only in KO mice at 3 weeks after injury.

Adiponectin Deficiency Increases Proliferation of Vascular Smooth Muscle Cells in Injured Arteries—Next we assessed proliferation of vascular smooth muscle cells (VSMCs) by immunohistochemical detection of BrdUrd-labeled VSMCs in the sections from each femoral artery at 2 weeks after injury (Fig. 2A). Quantitative data of proliferation index revealed that intimal VSMC proliferation induced by vascular injury was ~2-fold greater in KO mice than in WT mice (p = 0.001) (Fig. 2B). In non-injured arteries of both WT and KO mice, BrdUrd-labeled VSMCs were barely detectable (data not shown). These data demonstrate that deficiency of adiponectin in KO mice caused severe neointimal hyperplasia after artery injury, suggesting an inhibitory effect of adiponectin on the proliferation of VSMCs in injured arteries.

Adenovirus-mediated Supplement of Adiponectin Attenuates Neointimal Thickening in Injured Arteries—To investigate the in vivo effects of adiponectin supplement on neointimal hyper-
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In vitro experiments provided strong evidence that adiponectin exerts suppressive effects on VSMC proliferation. PDGF, HB-EGF, basic FGF, and EGF have potent mitogenic activities on HASMCs. Adiponectin treatment attenuated growth factor-induced DNA synthesis in HASMCs (Fig. 4A). The inhibitory effect of adiponectin on HASMC proliferation induced by HB-EGF was directly shown by counting the cell number (Fig. 4B). In addition, adiponectin also suppressed HB-EGF-induced migration of HASMCs (Fig. 4C).

Adiponectin Attenuates the Expression of HB-EGF mRNA in Cultured Endothelial Cells—Next we investigated whether adiponectin could suppress the production of HB-EGF in endothelial cells. Adiponectin treatment completely blocked the TNFα-mediated increase of HB-EGF mRNA in HAEcs (Fig. 5).

**DISCUSSION**

In the current study, we demonstrated that adiponectin-null mice exhibited augmented intimal proliferation in mechanically injured vascular walls. Adenovirus-mediated supplement of adiponectin improved the intimal thickening in KO mice to the WT level. How does adiponectin suppress intimal thickening? Fig. 6 illustrates a working model based on the results of our in vivo and in vitro experiments described in the present study. Adiponectin suppressed the expression of HB-EGF in stimulated endothelial cells of injured vascular wall and also the proliferation and migration of smooth muscle cells stimulated by various growth factors such as PDGF, basic FGF, EGF, and HB-EGF. These suppressive effects of adiponectin on the production and action of growth factors in vascular wall should explain the mechanism for the suppressive action of adiponectin on the vascular stenosis and indicate that it could prevent adiponectin synthesis.
injury-induced intimal thickening.

Plasminogen activator inhibitor-1 and HB-EGF are vasoactive substances produced by adipose tissue, although these substances are not adipose-specific. Both factors are considered to promote the development of vascular diseases in obesity (6, 7). Contrary to these factors, the plasma concentration of adipose-specific adiponectin is lower in obese subjects and patients with coronary artery disease (11, 12). The present study demonstrated in vivo and in vitro that adiponectin suppressed VSMC proliferation. Taken together, adipose tissue secretes both the offense molecules (plasminogen activator inhibitor-1 and HB-EGF) and the defense molecule (adiponectin) into the blood stream, reaching the vascular wall. Then, in obesity, both the increase of offense molecules and decrease of defense molecule(s) in plasma should aggravate vascular diseases. Considering the adipose specificity, adiponectin should play a major role in the adipo-vascular axis.

Recent studies have identified the role of various molecules derived from adipose tissue in the development of insulin resistance. These include TNFα, leptin, and resistin (3–5). More recently, adiponectin treatment has been shown to improve fatty acid oxidation and insulin resistance in diabetic animals (23, 24). Adiponectin-null mice show normal adiponectin sensitivity under a regular diet but severe insulin resistance under a high fat/high sucrose diet (17). Interestingly, subjects carrying a missense mutation in the adiponectin gene associated with hypo-adiponectinemia exhibit the phenotype of the metabolic syndrome, including insulin resistance and coronary artery disease (25). These findings suggest that hypo-adiponectinemia associated with obesity is located upstream of metabolic syndrome in the pathophysiology. In the present study, adiponectin-null mice showed profound neointimal hyperplasia despite normal glucose and lipid metabolism. Our results indicate that injury-induced neointimal formation does not accelerate as a result of abnormalities of glucose and lipid metabolism but is directly caused by adiponectin deficiency. Therapeutic approaches that increase plasma adiponectin concentration could be useful in preventing restenosis after vascular intervention.

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REFERENCES
1. Spiegelman, B. M., and Flier, J. S. (2001) Cell 104, 531–543
2. Friedman, J. M. (2000) Nature 404, 632–634
3. Friedman, J. M., and Halaas, J. L. (1998) Nature 395, 763–770
4. Hotamisligil, G. S., Shargill, N. S., and Spiegelman, B. M. (1993) Science 259, 87–91
5. Stepan, C. M., Bailey, S. T., Bhat, S., Brown, E. J., Banerjee, R. R., Wright, C. M., Patel, H. R., Ahima, R. S., and Lazar, M. A. (2001) Nature 409, 307–312
6. Shimomura, I., Funahashi, T., Takahashi, M., Maeda, K., Kotani, K., Nakamura, T., Yamashita, S., Miura, M., Fukuda, Y., Takenaka, K., Tokunaga, K., and Matsuzawa, Y. (1996) Nat. Med. 2, 800–803
7. Matsunoto, S., Kishida, K., Shimomura, I., Maeda, N., Nagaretani, H., Matsuda, M., Nishizawa, H., Kihara, S., Funahashi, T., and Matsuzawa, Y. (2002) Biochem. Biophys. Res. Commun. 292, 781–786
8. Maeda, K., Obukuro, K., Shimomura, I., Funahashi, T., Matsuzawa, Y., and Matsubara, K. (1996) Biochem. Biophys. Res. Commun. 221, 286–289
9. Scherer, P. E., Williams, S., F支援, M., Baldini, G., and Lodish, H. F. (1995) J. Biol. Chem. 270, 26746–26749
10. Hu, E., Liang, P., and Spiegelman, B. M. (1996) J. Biol. Chem. 271, 10703–10703
11. Arita, Y., Kihara, S., Ouchi, N., Takahashi, M., Maeda, K., Miyagawa, J., Hotta, K., Shimomura, I., Nakamura, T., Miyaoaka, K., Kuriyama, H., Nishida, M., Yamashita, S., Obukuro, K., Matsubara, K., Muraguchi, M., Oohoto, Y., Funahashi, T., and Matsuzawa, Y. (1999) Biochem. Biophys. Res. Commun. 257, 79–83
12. Ouchi, N., Kihara, S., Arita, Y., Maeda, K., Kuriyama, H., Okamoto, Y., Hotta, K., Nishida, M., Takahashi, M., Nakamura, T., Yamashita, S., Funahashi, T., and Matsuzawa, Y. (1999) Circulation 100, 2473–2476
13. Zoccali, C., Mallamaci, F., Truppo, G., Benedetto, F. A., Cutrupi, S., Parlongo, S., Malatino, L. S., Bonanno, G., Seminara, G., Rapisarda, F., Fatazzu, P., Benui, M., Nicola, G., Tanaka, S., Ouchi, N., Kihara, S., Funahashi, T., and Matsuzawa, Y. (2002) J. Am. Soc. Nephrol. 13, 134–141
14. Okamoto, Y., Arita, Y., Nishida, M., Muraguchi, M., Ouchi, N., Takahashi, M., Ijura, T., Inui, Y., Kihara, S., Nakamura, T., Yamashita, S., Miyagawa, J., Inagaki, T., and Matsuzawa, Y. (2000) Horm. Metab. Res. 32, 47–50
15. Ouchi, N., Kihara, S., Arita, Y., Okamoto, Y., Maeda, K., Kuriyama, H., Hotta, K., Nishida, M., Takahashi, M., Muraguchi, M., Oohoto, Y., Nakamura, T., Yamashita, S., Funahashi, T., and Matsuzawa, Y. (2000) Circulation 102, 1309–1310
16. Ouchi, N., Kihara, S., Arita, Y., Nishida, M., Matsuyama, M., Oohoto, Y., Ishigami, M., Kuriyama, H., Kishida, K., Nishizawa, H., Hotta, K., Muraguchi, M., Oohoto, Y., Yamashita, S., Funahashi, T., and Matsuzawa, Y. (2001) Circulation 103, 1057–1063
17. Maeda, N., Shimomura, I., Kishida, K., Nishizawa, H., Matsuda, M., Nagaretani, H., Furuyama, N., Kondo, H., Takahashi, M., Arita, Y., Komuro, R., Ouchi, N., Kihara, S., Tochino, Y., Okutomi, K., Horie, M., Takeda, S., Aoyama, T., Funahashi, T., and Matsuzawa, Y. (2002) Nat. Med. 8, 731–737
18. Sata, M., Masejima, Y., Adachi, F., Fukino, K., Sairiya, A., Sugira, S., Aoyagi, T., Imai, Y., Kurihara, H., Kinuma, K., Oota, M., Makuchii, M., Hirata, Y., and Nogai, R. (2000) J. Mol. Cell. Cardiol. 32, 2097–2104
19. Sata, M., Sairiya, A., Kunito, A., Tojo, A., Okada, S., Tokunsha, T., Hirai, H., Makuchii, M., Hirata, Y., and Nogai, R. (2002) Nat. Med. 8, 403–409
20. Karas, R. H., Schulten, H., Pare, G., Aronovitz, M. J., Ohlsson, C., Gustafsson, J. A., and Mendelsohn, M. E. (2001) Circ. Res. 89, 534–539
21. Hishikawa, K., Omae, B. S., Tanner, F. C., Nakaki, T., Fujii, T., and Lucher T. F. (1999) Circulation 100, 2108–2112
22. Yu, S.-M. Tsai, S.-Y., Gih, J.-H., Ko, F.-N., Teng, C.-M., and Ou, J. T. J. (1996) Circulation 94, 547–554
23. Yamauchi, T., Kamon, J., Waki, H., Terashita, Y., Kubota, N., Hara, K., Morii, Y., Ide, T., Murakami, K., Tesbyoya-Kasukawa, N., Ezaki, O., Akanuma, Y., Gavrilova, O., Vinson, C., Reitman, L. M., Kagechika, H., Shudo, K., Yoda, M., Nakano, T., Tohe, K., Nogai, R., Kimura, S., Tomita, M., Froguel, P., and Kodawaki, T. (2001) Nat. Med. 7, 941–946
24. Berg, A. H., Combe, T. P., Du, X., Brewlee, M., and Scherer, P. E. (2001) Nature Med. 7, 947–953
25. Kondo, H., Shimomura, I., Matsuzawa, Y., Kumada, M., Takahashi, M., Matsuda, M., Ouchi, N., Kihara, S., Kawamoto, T., Sumitoku, S., Funahashi, T., and Matsuzawa, Y. (2002) Diabetes 51, 2325–2328