Protein Region Important for Regulation of Lipid Metabolism in Angiopoietin-like 3 (ANGPTL3)

ANGPTL3 IS CLEAVED AND ACTIVATED IN VIVO*

Received for publication, March 20, 2003, and in revised form, July 21, 2003
Published, JBC Papers in Press, August 8, 2003, DOI 10.1074/jbc.M302861200

Mitsuru Ono‡§, Tetsuya Shimizugawa§, Mitsuru Shimamura‡, Kenichi Yoshida‡‡, Chisa Noji-Sakikawa‡, Yosuke Ando‡‡, Ryuta Koishi‡, and Hidehiko Furukawa‡

From the ‡Biomedical Research Laboratories, the §Pharmacology and Molecular Biology Research Laboratories, Sankyo Co., Ltd., 2-58 Hiromachi 1-chome, Shinagawa-ku, Tokyo 140-8710 and the ††Medical Safety Research Laboratories, Sankyo Co., Ltd., 717, Horikoshi, Fukuroi, Shizuoka 437-0065, Japan

Angiopoietin-like 3 (ANGPTL3) is a secreted protein that is expressed predominantly in the liver (1). ANGPTL3 is structurally similar to angiopoietins (2–4), which are vascular endothelial growth factors. It also has a signal peptide for secretion, a helical domain predicted to form coiled-coils (CCD), and a C-terminal globular fibrinogen-like domain (FLD). Angiopoietins are ligands that bind to the receptor tyrosine kinase Tie2. The FLDs of angiopoietins bind to Tie2, and the CCDs of angiopoietins mediate ligand homo-oligomerization (5). Although ANGPTL3 does not bind to Tie2, it may also induce angiogenesis by binding to integrin αβ3 (6).

We reported that KK/Snk (previously, KK/San) had abnormally low plasma lipids and identified a mutation in the Angptl3 gene as the cause of the hypolipidemic phenotype of KK/Snk mice (7, 8). Injection of recombinant ANGPTL3 or adenoviruses encoding ANGPTL3 resulted in an increase in plasma lipids in mice. It has been speculated that ANGPTL3 was capable of inhibiting lipolysis of triglycerides, because an injection of recombinant ANGPTL3 induced a rapid increase in mouse plasma triglyceride levels (9, 10). We further reported that very low density lipoprotein clearance was enhanced in KK/Snk mice and that recombinant ANGPTL3 directly inhibited lipoprotein lipase activity (LPL) activity in vitro (11). These observations suggest that ANGPTL3 could be involved in the regulation of lipid metabolism by inhibiting LPL activity.

Thus, ANGPTL3 seems to be a multifunctional factor that may regulate both lipid metabolism and angiogenesis. The C-terminal FLD of ANGPTL3 was reported to be involved in angiogenesis via binding to integrin αβ3 (6), but the domain of ANGPTL3 that is involved in the regulation of lipid metabolism has not been elucidated.

In this study, we constructed various mutants of ANGPTL3 and analyzed the region of ANGPTL3 required for its function in lipid metabolism. Further, as it was revealed that ANGPTL3 was cleaved in the linker region between CCD and FLD, we estimated the importance of this cleavage to the activity of ANGPTL3 in vivo.

EXPERIMENTAL PROCEDURES

Construction of Mutants—We inserted each mutant DNA into a mammalian expression vector (pMIE88) as described below. Full-length ANGPTL3 cDNA was cloned previously (8). The cDNA was amplified by polymerase chain reaction (PCR) using the sense primer, 5′-TTGAATTCGGCTTGCCACCATGGTCACAATTAAGCTCCTTCTTTATTTG-3′ (includes an EcoRI site and a Kozak consensus sequence, underlined), and the antisense primer, 5′-GTCTAGACCTCTGTCATCTCAAAAGCTTCTTTATTTG-3′ (includes an XbaI site, underlined), digested with EcoRI and XbaI, and cloned into the corresponding sites of pMIE88 to form pME/ANGPTL3. Two synthesized oligonucleotides, 5′-ATGAGCAGATCCAGGATGAGGTGAGTGGGGA-3′ and 5′-ATGAGCAGATCCAGGATGAGGTGAGTGGGGA-3′, were annealed to each other and cloned into the BclI site of pME/ANGPTL3, which is located near the end of the signal peptide-coding region, to form pME/His/ANGPTL3. Plasmid pME/ANGPTL3-(207–460) was generated from pME/ANGPTL3 by digestion with BclI and SpeI, purification of a 3.8-kbp fragment, filling in of the 5′ overhang with T4 DNA polymerase, and self-ligation. Plasmid pME/ANGPTL3-(79–207) was generated from pME/ANGPTL3-(17–207) by digestion with BclI, purification of a 4.2-kbp fragment, and self-ligation. Plasmid pME/ANGPTL3-(17–165) was cleaved at two sites, Arg221 and Arg224, and Arg224 and Thr225, respectively.

These findings suggest that the cleavage of ANGPTL3 is important for the activation of ANGPTL3 in vivo.
was generated from pMEHis/ANGPTL3 by replacing the EcoRI/SpeI fragment with an EcoRI/SpeI fragment that was generated by PCR using the sense primer 5'-TAAACGTCCGAGATCGGCTGCCG-3' and the antisense primer 5'-ACACTCTTTTATTAAGGTATCCCTGG-3'. Plasmid pME/ANGPTL3-(17–207mu) was generated from pME/ANGPTL3-(17–207). Substitutions of H62I, K63N, and K65N were introduced by site-directed mutagenesis using a synthetic oligonucleotide 5'-TCTAAACACACAGCGCAGCCTTTAATGACG-3'. Plasmid pME/His/ANGPTL3-(5Rmu) was generated from pME/His/ANGPTL3. To perform the substitutions of R204T and R205T, the EcoRI/SpeI fragment was replaced with the EcoRI/SpeI fragment produced by PCR using the sense primer 5'-TAAACGTCCGAGATCGGCTGCCG-3' and the antisense primer 5'-GTCTATACACACCGAGGCGCAAATTTAATGACG-3'. Plasmid pME/His/ANGPTL3-(5Rmu) was generated from pME/His/ANGPTL3. To perform the substitutions of R204T and R205T, the EcoRI/SpeI fragment was replaced with the EcoRI/SpeI fragment produced by PCR using the sense primer 5'-TAAACGTCCGAGATCGGCTGCCG-3' and the antisense primer 5'-GTCTATACACACCGAGGCGCAAATTTAATGACG-3'.

Construction of Recombinant Adenoviruses—Recombinant adenoviruses were generated as previously described (12) using an adenovirus expression vector kit (Takara Shuzo). Co-administration of the Cre recombinase and the adenovirus encoding the desired transgenic protein was performed by a 3-step transduction process. In the first step, a Cre recombinase-expressing adenovirus was injected into the mice. In the second step, a transgenic adenovirus encoding the desired transgenic protein was injected into the mice. In the third step, an adenovirus expressing the Cre recombinase was injected into the mice. The adenovirus expressing the Cre recombinase was used to activate the transgenic protein.

Characterization of the Cleavage Site of ANGPTL3—ANGPTL3 is a protein that is expressed in the liver and is involved in lipid metabolism. It is known to be involved in the regulation of triglyceride levels in mice. To determine the region of ANGPTL3 involved in lipid metabolism, we first tested whether the N-terminal CCD-containing region or the C-terminal FLD-containing region, alone, could increase plasma triglyceride levels in mice. We constructed deletion mutants of ANGPTL3, ANGPTL3-(17–207) and ANGPTL3-(207–460) (Fig. 1A) and generated recombinant adenoviruses, Ad/ANGPTL3-(17–207) and Ad/ANGPTL3-(207–460). After confirming that recombinant adenoviruses were released in the supernatant of the adenovirus-infected HeLa cells (Fig. 1B), the adenoviruses were injected into KK/Snk mice. The recombinant adenoviruses were also detected in the plasma of mice injected with Ad/ANGPTL3-(17–207) and Ad/ANGPTL3-(207–460) (Fig. 1C).

RESULTS

N-terminal Fragment of ANGPTL3 Increases Plasma Triglyceride Levels in Mice: Determination of the Region Essential for Activity—To determine the region of ANGPTL3 involved in lipid metabolism, we first tested whether the N-terminal CCD-containing region or the C-terminal FLD-containing region, alone, could increase plasma triglyceride levels in mice. We constructed deletion mutants of ANGPTL3, ANGPTL3-(17–207) and ANGPTL3-(207–460) (Fig. 1A) and generated recombinant adenoviruses, Ad/ANGPTL3-(17–207) and Ad/ANGPTL3-(207–460). After confirming that recombinant adenoviruses were released in the supernatant of the adenovirus-infected HeLa cells (Fig. 1B), the adenoviruses were injected into KK/Snk mice. The recombinant adenoviruses were also detected in the plasma of mice injected with Ad/ANGPTL3-(17–207) and Ad/ANGPTL3-(207–460) (Fig. 1C). The plasma triglyceride levels of Ad/ANGPTL3-(17–207)–injected mice were dramatically increased, whereas those of Ad/ANGPTL3-(207–460)–injected mice were not (Fig. 1D). To narrow down the essential region of the functional domain of ANGPTL3, we generated recombinant adenoviruses, Ad/ANGPTL3-(17–165) and Ad/ANGPTL3-(79–207) and injected them into KK/Snk mice. Injection of Ad/ANGPTL3-(17–165) resulted in an increase in plasma triglyceride levels, whereas injection of Ad/ANGPTL3-(79–207) did not (Fig. 1E). Although the amount of recombinant proteins secreted from Ad/ANGPTL3-(79–207)-infected HeLa cells was greater than that from Ad/ANGPTL3-(17–165)-infected HeLa cells (Fig. 1B), we could not detect any recombinant proteins in the plasma of mice injected with Ad/ANGPTL3-(79–207) (Fig. 1C, lane d). These data show that the region 17–165 elicits activity and suggest that the region 17–78 could be essential for protein stability in vivo. To evaluate whether a shorter fragment consisting of the first 78 N-terminal amino acid residues and not including the coiled-coil domain was active or not, we constructed an expression vector that encoded 1–78 of ANGPTL3 and transfected it into COS-1 cells. However, we could not detect any recombinant protein in the supernatant of the COS-1 cells (data not shown). Therefore, we could not elucidate whether region 17–78 was sufficient for the activity or not. On the other hand, we found that there was a putative heparin-binding motif (14), 61VHKTGK66, in the region 17–78. To analyze the importance of this motif, we constructed a mutant in which basic amino residues of the motif VHKTGG were substituted with VINTNG (ANGPTL3-(17–207mu)) (Fig. 2A) and generated an adenovirus Ad/ANGPTL3-(17–207mu). Injection of Ad/ANGPTL3-(17–207mu) into KK/Snk mice did not increase plasma triglyceride levels (Fig. 2B) even though recombinant proteins were detected in the plasma of Ad/ANGPTL3-(17–207mu)-infected mice as much as in the plasma of Ad/ANGPTL3-(17–207)-infected mice (Fig. 2C), where plasma triglyceride levels were dramatically increased (Fig. 2B). To elucidate the LPL inhibitory activity of ANGPTL3-(17–207) and ANGPTL3-(207–460) in vitro, we prepared purified proteins that were deacetylated in 10 μM diithiothreitol (Sigma) and alkylated in 55 mM iodoacetamide (Wako Pure Chemical Co.). The deacetylated and alkylated proteins were digested with lysyl endopeptidase (Wako Pure Chemical Co.) or deglycosylated with PNGase F (New England Biolabs, Beverly, MA) followed by digestion with sequencing grade endoproteinase Glu-C (Promega, Madison, WI). Then, formic acid was added to the peptides (final concentration 0.5%), and the digested proteins were analyzed by liquid chromatography electrospray ionization mass spectrometry: column, original fused silica electrospray needle packed with Inertsil ODS-2 (GL Science, Tokyo, Japan); solvent delivery, CapLC System (Waters, Milford, MA); solvent A, 0.05% formic acid/H2O; solvent B, 0.05% formic acid/acetonitrile; linear gradient elution, 0–30% solvent B over 1 h; mass spectrometer, Q-ToF (Micromass, Manchester, UK) with an electrospray ion source. The resulting spectra were processed with ProteinLynx (Micromass, Manchester, UK) software.
As shown in Fig. 2D, recombinant ANGPTL3-(17–207) inhibited the lipolysis of triglycerides by LPL. ANGPTL3-[17–207mu] also inhibited lipolysis but only at high concentrations. The inhibitory effect of ANGPTL3-[17–207mu] on LPL in vitro was much lower than that of ANGPTL3-(17–207).

**ANGPTL3 Is Cleaved in Vivo in Human and Mouse**—As mentioned above, it was shown that the N-terminal region of ANGPTL3 was involved in lipid metabolism, whereas the C-terminal region of ANGPTL3 was involved in angiogenesis. We could not detect any ANGPTL3 protein in human plasma by Western blotting using either anti-ANGPTL3 polyclonal or monoclonal antibodies (data not shown). To detect ANGPTL3 protein in human plasma and determine whether ANGPTL3 can be processed into smaller fragments, we purified ANGPTL3 from human plasma by affinity chromatography using anti-ANGPTL3 monoclonal antibody that recognizes the N-terminal 17–207 of ANGPTL3. Purified ANGPTL3 was visualized by Western blotting using anti-ANGPTL3 polyclonal antibody. We detected cleaved ANGPTL3 as well as full-length ANGPTL3 (Fig. 3A).

In addition, we detected cleaved proteins, whose molecular masses were ~30–35 kDa, as well as full-length proteins in the plasma of Ad/His/ANGPTL3-injected mice (Fig. 3B). Then, we purified the N-terminal fragment of ANGPTL3 from the plasma of Ad/His/ANGPTL3-injected mice by nickel affinity chromatography. We digested the purified protein sample with lysyl endopeptidase or endoproteinase Glu-C and analyzed the digested fragments by liquid chromatography electrospray ionization mass spectrometry. As a result, we detected peptides 198EIENQLRRTSIQEPTEISLSSKPR221 and 198EIENQLRRTSIQEPTEISLSSKPRAPR224 in the sample digested with lysyl endopeptidase, and peptides 214ISLSSKPR221 and 214ISLSSKPRAPR224 in the sample digested with endoproteinase Glu-C. Thus, it was found that ANGPTL3 was cleaved at two sites, Arg221 and Arg224, in the linker region between CCD and FLD (Fig. 3C).

**Cleavage of ANGPTL3 Is Important for Its Activation in Vivo**—We showed that ANGPTL3 was cleaved in mice and that the N-terminal fragment of ANGPTL3 increased plasma triglyceride levels. Although full-length ANGPTL3 inhibited lipolysis of triglycerides by LPL in vitro to the same extent as the N-terminal fragment of ANGPTL3, we considered that cleavage of ANGPTL3 could be required to increase plasma triglyceride levels. To evaluate the effect of the cleavage, we generated a recombinant adenovirus (Ad/His/ANGPTL3-[5Rmu]) that encoded a mutant ANGPTL3 in which all five arginine residues located in and near the linker region between the CCD and FLD were substituted with other amino acids (R203T, R204T, R221S, R224S, and R235S) (Fig. 4A). We injected Ad/His/ANGPTL3-[5Rmu] into KK/Snk mice. We confirmed that cleaved recombinant proteins were not detected in the plasma.
of Ad/His/ANGPTL3-[5Rmu]-injected mice (Fig. 4B). This result suggests that the substitution of these amino acids alters the susceptibility to cleavage in vivo. At 1 day after injection of the recombinant adenovirus, the plasma triglyceride concentration in Ad/His/ANGPTL3-[5Rmu]-injected mice was 186.4 ± 11.2 mg/dl/deciliter (n = 3), whereas that of Ad/His/ANGPTL3- and Ad/ANGPTL3-(17–207)-injected mice was dramatically increased and reached 1243 ± 184 and 2564 ± 67 mg/dl/deciliter, respectively (Fig. 4C). Recombinant proteins were detected in the plasma of Ad/His/ANGPTL3-[5Rmu]-injected mice as much as in the plasma of Ad/His/ANGPTL3- and Ad/ANGPTL3-(17–207)-injected mice (Fig. 4B). These results indicate that His/ANGPTL3-[5Rmu] is less active than His/ANGPTL3 in mice. Next, we prepared recombinant His/ANGPTL3 and His/ANGPTL3-[5Rmu] and confirmed that recombinant His/ANGPTL3-[5Rmu] inhibited lipolysis of triglycerides by acting on LPL as potently as recombinant His/ANGPTL3 (Fig. 4D). These results indicate that the substitution of the five amino acids did not result in the loss of lipolysis inhibition in vitro and that cleavage of ANGPTL3 is an important process for the activation of ANGPTL3 in vivo.

DISCUSSION

ANGPTL3 consists of the N-terminal CCD, C-terminal FLD, and a linker region. We have shown that ANGPTL3 is cleaved in the linker region between CCD and FLD in vivo and that the cleaved N-terminal portion of ANGPTL3 inhibits LPL activity and increases the level of circulating plasma triglycerides in mice. Furthermore, a cleavage-resistant mutant of ANGPTL3 was found to be less active than the cleavable wild-type ANGPTL3 in increasing mouse plasma triglyceride levels, which suggests that cleavage of ANGPTL3 is an important
We showed that the N-terminal 17–207 of ANGPTL3 has the ability to regulate lipid metabolism. It was reported that the C-terminal portion of ANGPTL3 had the ability to induce angiogenesis (6). Therefore, the regions of ANGPTL3 involved in angiogenesis and lipid metabolism were determined to be different from each other. Furthermore, we were able to narrow the region involved in lipid metabolism down to the region 17–165. The plasma triglyceride levels of mice injected with Ad/ANGPTL3-(17–165) (1304 ± 262 mg/deciliter at 1 day postinjection) were lower than those injected with Ad/ ANGPTL3-(17–207) (5056 ± 93 mg/deciliter at 1 day postinjection) (Fig. 1, D and E). However, these plasma triglyceride levels seemed to be correlated with the plasma levels of recombiant protein (Fig. 1C). These results suggest that the region 17–165 could increase the plasma triglyceride levels as well as the region 17–207.

We also showed that a substitution of basic amino residues in the putative heparin-binding motif located at 61–66 of the N-terminal resulted in a dramatic decrease in its inhibitory activity on LPL in vitro. Although it was reported that heparin modulated LPL activity in vitro (15), only adding heparin did not affect the inhibitory effect of ANGPTL3 on LPL activity (data not shown). We reported that ANGPTL4 could also inhibit LPL activity (16), but we could not find a putative heparin-binding motif in the active region of ANGPTL4. These findings suggest that an area containing the putative heparin-binding motif may interact with a factor involved in lipolysis other than heparin, and the binding of this factor to the putative heparin-binding motif may be important for the activity. To elucidate how ANGPTL3 inhibits the lipolysis of triglyceride, it must be determined whether ANGPTL3 directly inhibits LPL or whether another factor is involved. A putative heparin-binding motif-substituted mutant protein would be useful as a negative control in further studies.

With regard to the cleaved N-terminal fragment, we detected cleaved ANGPTL3 in human and ANGPTL3-overexpressed mice. Although we did not use any protein inhibitors when collecting plasma, we did confirm that the recombinant full-length ANGPTL3 protein was not cleaved during incubation with mouse plasma at 37 °C for an hour. We also considered that the cleavage of ANGPTL3 that we observed did not occur after we collected the plasma. We determined that the main cleavage sites were at Arg221/Ala222 and Arg224/Thr225, but other small bands of cleaved proteins were also detected (Fig. 3B), suggesting that ANGPTL3 may have been cleaved at other sites albeit only a little. To examine this further, we substituted five arginine residues located in and near the linker region between CCD and FLD. We confirmed that the substitution of these five amino acids did not alter the LPL inhibitory activity of these recombinant proteins in vitro (Fig. 4D). We demonstrated that the increase of plasma triglyceride levels in the cleavage-resistant mutant ANGPTL3-overexpressed mice was far less than that in the wild-type ANGPTL3-overexpressed mice (Fig. 4C), even though the amounts of recombinant protein detected in the plasma of the cleavage-resistant mutant ANGPTL3-overexpressed mice were greater than those of the wild-type ANGPTL3-overexpressed mice (Fig. 4B). These results indicate that non-cleavable, full-length ANGPTL3 is less active in increasing triglyceride levels in vivo despite its high LPL inhibitory activity in vitro.

There could be two explanations behind this inconsistency between the in vitro and in vivo activities of full-length ANGPTL3. One is the possibility that ANGPTL3 inhibition of LPL is not the cause of the increase in triglyceride levels in vivo.

Fig. 4. Importance of ANGPTL3 cleavage for the activity in vivo. A, cleavage sites of ANGPTL3 (arrowhead) and the five substituted amino acid residues (arrow) in His/ANGPTL3-[5Rmu]. KK/Snk mice were each intravenously injected with 200 μl of phosphate-buffered saline (n = 3) or 2.5 × 10⁸ pfu/ml Ad/His/ANGPTL3 (n = 3), Ad/His/ANGPTL3-[5Rmu] (n = 3), and Ad/ANGPTL3-(17–207) (n = 2). B, recombinant proteins in 0.25 μl of plasma at 1 day after injection of Ad/His/ANGPTL3 (lanes 1–3), Ad/His/ANGPTL3-[5Rmu] (lanes 4–6), and Ad/ANGPTL3-(17–207) (lanes 7 and 8) were detected by Western blot analysis using polyclonal anti-ANGPTL3 antibody. C, plasma triglyceride levels before (Pre) and at 1, 2, 4, 7, 11, and 14 days after injection. Error bars indicate S.E. D, effect of recombinant proteins on LPL activity. Error bars indicate S.E. (n = 3).
but that the increase in triglyceride levels is due to ANGPTL3 affecting hepatic lipase. The other is the possibility that full-length ANGPTL3 is not able to inhibit LPL in vivo unless it is cleaved.

With regard to the first hypothesis, we had found that the inhibition of hepatic lipase by ANGPTL3-(17–207) was very weak compared with that of LPL (data not shown). Moreover, when the N-terminal fragments of ANGPTL3 (ANGPTL3-(17–207)) and its mutant (ANGPTL3-(17–207mu)) were tested, the inhibitory activity on LPL in vitro corresponded well to the triglyceride-increasing activity in vivo. Therefore, we considered that the triglyceride increase was because of the inhibition of LPL by ANGPTL3.

With regard to the second hypothesis, it is known that some growth factors and cytokines are associated with other proteins that can suppress their actions and that in order to activate these factors and cytokines, they must first be liberated from these proteins by proteolytic processing (17–19). Natural full-length ANGPTL3 may also be in complex with a protein, and this protein may be bound to the active site of ANGPTL3. It could be considered that the cleavage of ANGPTL3 dislodges the protein and releases the active ANGPTL3 fragment. We previously found that ANGPTL3 was able to bind to adipocytes and heparin was partially able to inhibit this binding (20). These observations suggest that ANGPTL3 may bind to proteoglycans. Therefore, proteoglycans could be the proteins binding to ANGPTL3 and suppressing its activity.

ANGPTL3 is mainly expressed in the liver (1). In plasma, full-length ANGPTL3 was also detected (Fig. 3, A and B). These findings let us speculate that after being secreted from the liver, ANGPTL3 circulates in the blood in the full-length form and carries out its role in lipogenesis at the target site upon its cleavage.

Acknowledgments—We thank K. Maruyama for providing the pME18S vector, T. Tanimoto, K. Miura, and H. Yasumo for assistance with nucleotide sequencing, and T. Kasaka and T. Matsuoka for kind advice on protein analysis. We are grateful to N. Nakamura, J. Ohsumi, K. Kohama, T. Inaba, and T. Koga for helpful discussions.

REFERENCES

1. Conklin, D., Gilbertson, D., Taft, D. W., Maurer, M. F., Whitmore, T. E., Smith, D. L., Walker, K. M., Chen, L. H., Wattler, S., Nehls, M., and Lewis, K. B. (1999) Genomics 2, 477–482.
2. Maisonspierre, P. C., Suri, C., Jones, P. F., Bartunkova, S., Wiegand, S. J., Radziejewski, C., Compton, D., McClain, J., Aldrich, T. H., Papadopoulos, N., Daly, T. J., Davis, S., Sato, T. N., and Yancopoulos, G. D. (1999) Science 277, 55–60.
3. Davis, S., Aldrich, T. H., Jones, P. F., Achenon, A., Compton, D. L., Jain, V., Ryan, T. E., Bruno, J., Radziejewski, C., Maisonspierre, P. C., and Yancopoulos, G. D. (1999) Cell 87, 1161–1169.
4. Valenzuela, D. M., Griffiths, J. A., Rojas, J., Aldrich, T. H., Jones, P. F., Zhou, H., McClain, J., Copeland, N. G., Gilbert, D. J., Jenkins, N. A., Huang, T., Papadopoulos, N., Maisonspierre, P. C., Davis, S., and Yancopoulos, G. D. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 1904–1909.
5. Procopio, W. N., Pelavin, P. I., Lee, W. M. F., and Yelding, N. M. (1999) J. Biol. Chem. 274, 30196–30201.
6. Cameni, G., Papahar, M. T., Sherman, D., Kowalski, J., Nagel, M., Hass, P., Gunney, A., Bodary, S. C., Liang, X. H., Clark, R. B., Beresini, M. H., Ferrara, N., and Gerber, H.-P. (2002) J. Biol. Chem. 277, 17281–17290.
7. Shiraki, T., Yoshitaka, S., and Horikoshi, H. (1993) Diabetes Frontier 4, 641.
8. Koishi, R., Ando, Y., Ono, M., Shimamura, M., Yasuno, H., Fuijwara, T., Horikoshi, H., and Hidaka, F. (2002) Nat. Genet. 30, 151–157.
9. Rossetti, L., and Goldberg, I. J. (2002) Nat. Med. 8, 112–114.
10. Naoumova, R. P., and Betteridge, D. J. (2002) Lancet 359, 2215–2216.
11. Shimizuwa, T., Ono, M., Shimamura, M., Yoshida, K., Ando, Y., Koishi, R., Ueda, K., Inaba, T., Minekura, H., Kohama, T., and Furukawa, H. (2002) J. Biol. Chem. 277, 33742–33748.
12. Miyake, S., Makimura, M., Kanegae, Y., Harada, S., Sato, Y., Takamori, K., Tokuda, C., and Saito, I. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 1230–1234.
13. Kanegae, Y., Makimura, M., and Saito, I. (1994) Jpn. J. Med. Sci. Biol. 47, 157–166.
14. Cardin, A. D., and Weintraub, H. J. (1989) Arteriosclerosis 9, 21–32.
15. Litthell, H., and Bokerg, J. (1977) Scand. J. Clin. Lab. Invest. 37, 551–561.
16. Yoshida, K., Shimizuwa, T., Ono, M., and Furukawa, H. (2002) J. Lipid Res. 43, 1770–1772.
17. Taipale, J., and Keski-Oja, J. (1997) PASEJ 11, 51–59.
18. Hooper, N. M., Karran, E. H., and Turner, A. J. (1997) Biochem. J. 321, 265–279.
19. Fowlkes, J. L., and Winkler, M. K. (2002) Cytokine Growth Factor Rev. 13, 277–287.
20. Shimamura, M., Matsuda, M., Kobayashi, S., Ando, Y., Ono, M., Koishi, R., Furukawa, H., Makishima, M., and Shimomura, I. (2006) Biochem. Biophys. Res. Commun. 301, 604–609.
Protein Region Important for Regulation of Lipid Metabolism in Angiopoietin-like 3 (ANGPTL3): ANGPTL3 IS CLEAVED AND ACTIVATED IN VIVO
Mitsuru Ono, Tetsuya Shimizugawa, Mitsuru Shimamura, Kenichi Yoshida, Chisa Noji-Sakikawa, Yosuke Ando, Ryuta Koishi and Hidehiko Furukawa

J. Biol. Chem. 2003, 278:41804-41809.
doi: 10.1074/jbc.M302861200 originally published online August 8, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M302861200

Alerts:
  • When this article is cited
  • When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 18 references, 8 of which can be accessed free at http://www.jbc.org/content/278/43/41804.full.html#ref-list-1