J. Biochem. 2009;146(6)881–886
doi:10.1093/jb/mvp135

FGF-1-Induced Reactions for Biogenesis of apoE-HDL are Mediated by Src in Rat Astrocytes

Tomo Nishida, Jin-ichi Ito, Yuko Nagayasu and Shinji Yokoyama*

Biochemistry, Nagoya City University Graduate School of Medical Sciences, Kawasumi 1, Mizuho-cho, Mizuho-ku, Nagoya 467-8601, Japan

Received July 8, 2009; accepted August 18, 2009; published online August 27, 2009

Fibroblast growth factor-1 (FGF-1) is released from astrocytes in stress and stimulates MEK/ERK and PI3K/Akt pathways in autocrine fashion to increase synthesis of cholesterol and 25-OH-cholesterol, and to induce transport and secretion of apoE, respectively. FGF-1-induced phosphorylation of Src, and phosphorylation of MEK, ERK and Ark was inhibited by Src inhibitors in rat astrocytes. Src inhibitors also suppressed FGF-1-induced increase of biosynthesis and release of cholesterol and increase of apolipoprotein E (apoE) secretion. The results were reproduced in rat astrocytoma cells transfected by rat apoE and in 3T3-L1 cells. Down-regulation of Src expression reduced FGF-1-induced phosphorylation of the signalling protein and subsequent reactions. Increase by FGF-1 of messages of apoE and HMG-CoA reductase was not influenced by Src inhibitors or by its down-regulation. We conclude that FGF-1 activates Src for activation of MEK/ERK and PI3K/Akt pathways, while Src may not be involved in enhancement of transcription of the cholesterol-related genes.

Key words: Astrocytes, apolipoprotein E, high density lipoprotein, fibroblast growth factor-1, Src.

Abbreviations: apo, apolipoprotein; CNS, Central nervous system; FGF-1, fibroblast growth factor-1; FGFR1, FGF receptor 1; HDL, high density lipoprotein; PKB, protein kinase B; siRNA, small interfering RNA; TCA, trichloroacetic acid; TLC, thin layer chromatography.

Central nervous system (CNS) is segregated from systemic blood circulation by blood brain barrier, which plasma lipoproteins do not cross, so that CNS uses its own intercellular lipid transport system by high-density lipoprotein (HDL) (*1*). Astrocytes are the main HDL supplier in CNS with apolipoprotein E (apoE) synthesized by astrocytes themselves (*2*, *3*) and extracellular apoA-I from unknown sources (*4*, *5*).

ApoE production increases in the brain in the cases of injury and damage, acutely and perhaps chronically as well, such as cerebral infarction, nerve and brain injury and their degeneration (*6–15*). We found that healing of the experimental cryo-injury of the brain was substantially retarded in the apoE-deficient mice (*16*). Production of fibroblast growth factor-1 (FGF-1) was observed in astrocytes in the peri-injury regions 2 days after the injury, both in the apoE-deficient and wild-type mice brain. ApoE production increased a few days later in the same regions of the wild-type mouse brain (*16*). FGF-1 is produced and released by astrocytes in culture and stimulates the astrocytes for apoE-HDL production (*17*, *18*). We further demonstrated that FGF-1 induces phosphorylation of the PI3K/Akt pathway for apoE transport and secretion and the phosphorylation of the MEK/ERK pathway for lipid biosynthesis via the FGF receptor(s) (*19*). FGF-1 stimulates MEK/ERK pathways also for production of 25-OH-cholesterol to activate LXRα for the apoE gene expression (*20*). On the other hand, c-Src, reportedly regulates physiological activities of FGF-1 such as proliferation of murine embryonic fibroblasts (*21*).

In the present work, we investigated the involvement of Src in an initial step(s) of the FGF-1-induced reactions of cholesterol biosynthesis, cholesterol release and apoE secretion and found that activation of Src protein is critically involved in activation of MEK/ERK and PI3K/Akt pathways in their upstream.

MATERIALS AND METHODS

Reagents—SU5402, an inhibitor of FGF receptor-1 (FGFR1) was purchased from Calbiochem. Src inhibitors (PP1 and SU6656) were obtained from BIOMOL and Calbiochem, respectively.

Preparation of Rat Astrocytes—Astrocytes were prepared from the 17-day-old fetal brain of Wistar rat according to the method described previously (*22*). Rat apoE/pcDNA3.his was transfected to transformed rat astrocyte GA-1 cells (*23*) that otherwise do not synthesize apoE (GA-1/25) (*19*). Mouse fibroblast 3T3-L1 cells were obtained from Riken Cell Bank. The cells were washed and incubated in 0.1% BSA/F-10 for 16 h before each experimental use.

Synthesis and Release of Cellular Cholesterol—To measure *de novo* synthesis of cholesterol, astrocytes were incubated with [^{14}C]-acetate (4 μCi/ml) for 2 h, lipid was

*To whom correspondence should be addressed.
Tel: +81-52-853-8139, Fax: +81-52-841-3480,
E-mail: syokoyam@med.nagoya-cu.ac.jp

extracted from the cells with hexane/isopropanol (3 : 2, v/v) and radioactivity was counted in cholesterol after separation by thin layer chromatography (TLC) (*22*). To determine cholesterol released into the medium, astrocytes were labelled by incubating with [^{14}C]-acetate (4 μCi/ml) for 24 h, washed three times and incubated in a fresh 0.02% BSA/F-10 for 6 h (*22*). Lipid was extracted from the medium with chloroform/methanol (2 : 1, v/v) and analysed by TLC to count radioactivity in cholesterol.

Analysis of Protein by Western blotting—The method was described previously (*19*). Protein in the conditioned medium, cytosol and membrane fraction was precipitated with 10% trichloroacetic acid (TCA) for the analysis of its 70 μg in 10% SDS–PAGE and immunoblotting with rabbit antibodies against rat apoE (a generous gift by Dr J. Vance, The University of Alberta), phosphorylated Akt (Thr-308) (Cell Signaling Technology), p44/42 MAP kinase (Cell Signaling Technology), phosphorylated MEK 1/2 (Ser217/221) (Cell Signaling Technology), MEK 1/2 (Cell Signaling Technology), mouse antibodies against protein kinase B (PKB)α/Akt (BD Transduction Laboratories), phosphorylated p44/p42 MAP kinase (Thr202/Tyr204) (Cell Signaling Technology), phosphorylated Src (Tyr416) (Upstate), Src (Upstate) and a goat antibody against FGF-1 (Santa Cluz Biotechnology).

Reverse Transcriptase Polymerase Chain Reaction—Total cellular RNA was isolated using ISOGEN (Nippon Gene) and subjected to reverse transcription to cDNA by Thermo Script (Invitrogen) with oligo-dT primers and amplification of cDNA by using the primers for apoE, HMG-CoA reductase, β-actin in a Gene Amp (Applied Biosystems). After an electrophoresis of the products in agarose gels, the bands were stained with EtBr solution (Nippon Gene Co. Ltd, Tokyo) and visualized by an ultraviolet transilluminator (UVP NML-20 E) at 302 nm. The primer pairs used were: 5′-ctgttggtcccattg ctgac-3′ (sense) and 5′-tgtgtgacttgggagctctg-3′ (antisense) for apoE; 5′-tgctgctttggctgtatgtc-3′ (sense) and 5′tgagcgtg aacaagaaccag-3′ (antisense) for HMG-CoA reductase. β-Actin primers were used as an internal control (*19*).

RNA Interference—Specific small interfering RNA (siRNA) for rat Src and universal control were obtained from Invitrogen and transfected into rat astrocytes using Lipofectamine RNAiMAX (Invitrogen) according to the manufacturer's protocol. After transfection, cells were harvested and subsequently mRNA expression and proteins were analyzed.

RESULTS

Signalling pathways by FGF-1 stimulation were investigated in rat astrocytes. Figure 1A demonstrates phosphorylation of the signal proteins MEK, ERK, Akt and Src. As reported previously (*19*), MEK, ERK and Akt proteins were phosphorylated by FGF-1 stimulation. In addition to those, Src protein was also phosphorylated by FGF-1 stimulation. These protein phosphorylation were all inhibited by an FGFR1 inhibitor SU5402 (Fig. 1A), showing that the reactions including Src phosphorylation were mediated by FGF-1 and its receptors. Figure 1B shows that phosphorylation of the signal proteins was inhibited by Src inhibitors, PP1 and SU6656.

FGF-1 induces signals of the PI3K/Akt pathway for apoE transport/secretion and the MEK/ERK pathway for lipid biosynthesis via the FGF receptor(s) (*19*, *24*). We therefore investigated involvement of Src in the FGF-1-induced cholesterol biosynthesis, cholesterol release and apoE secretion. Figure 1C demonstrates that Src inhibitors, SU6656 and PP1, inhibited cholesterol biosynthesis and its release induced by FGF-1. It is also shown that increase of apoE secretion by FGF-1 was inhibited by Src inhibitors. Thus, the findings were consistent with the effects of Src inhibitors on the MEK/ERK and PI3/Akt pathways, showing the involvement of Src phosphorylation in the upstream of signal activations in the FGF-1-induced reactions in astrocytes.

Involvement of Src in the signalling pathways was confirmed in the cell line cells. We used rat astrocyte cell line GA-1/25 cells, transformed rat astrocytes to which rat apoE/pcDNA3.his was transfected (*19*), and also mouse fibroblasts 3T3-L1. FGF-1-induced phosphorylation of MEK, ERK and Akt in GA-1/25 and 3T3-L1 cells (Fig. 2A and B). The phosphorylations in GA-1/25 cells were suppressed by Src inhibitors SU6656 and PP1, and those were inhibited by an FGF-1 receptor inhibitor SU5402 and by SU6656 and PP1 in 3T3-L1 cells (Fig. 2A and B). Secretion of apoE from GA-1/25 cells was increased by FGF-1 and the increase was reversed by SU6656 and PP1, indicating that Src is involved in the FGF-1-mediated reactions independent of transcriptional regulation of apoE (Fig. 2C). Cholesterol biosynthesis in GA-1/25 cells and its increase by FGF-1 were severely inhibited by SU6656 and PP1, indicating the presence of basic activation of Src in this cell line cells (Fig. 2D).

FGF-1 induces the increase of mRNA of the lipid-related genes such as apoE and HMG-CoA reductase, in time-dependent manners (Fig. 3A). Interestingly, neither Src inhibitors, SU6656 nor PP1, influenced these increases (Fig. 3B). Therefore, induction of these genes by FGF-1 does not involve Src activation, unlike induction of signalling pathways of MEK/ERK and PI3K/Akt for up-regulation of synthesis of cholesterol/25-OH-cholesterol (*20*) and enhancement of transport/secretion of apoE (*19*, *20*).

Involvement of Src in activation of the signalling pathways was further investigated by down-regulation of the Src gene using a specific siRNA. Figure 4A showed that down-regulation of the Src gene was saturated at 200 nM of siRNA so that this concentration was used thereafter. Src protein expression was almost completely suppressed in this condition (Fig. 4B). Phosphorylation of MEK, ERK and Akt were all reduced by the siRNA treatment of the cells (Fig. 4C). Under the same condition, the expression of the genes of apoE and HMG-CoA reductase was not significantly influenced at all (Fig. 4D).

DISCUSSION

ApoE is the major endogenous apolipoprotein in CNS, synthesized and secreted by astrocytes and microglias to form apoE-HDL (*1*). Production of apoE and apoE-HDL increases in response to acute and chronic

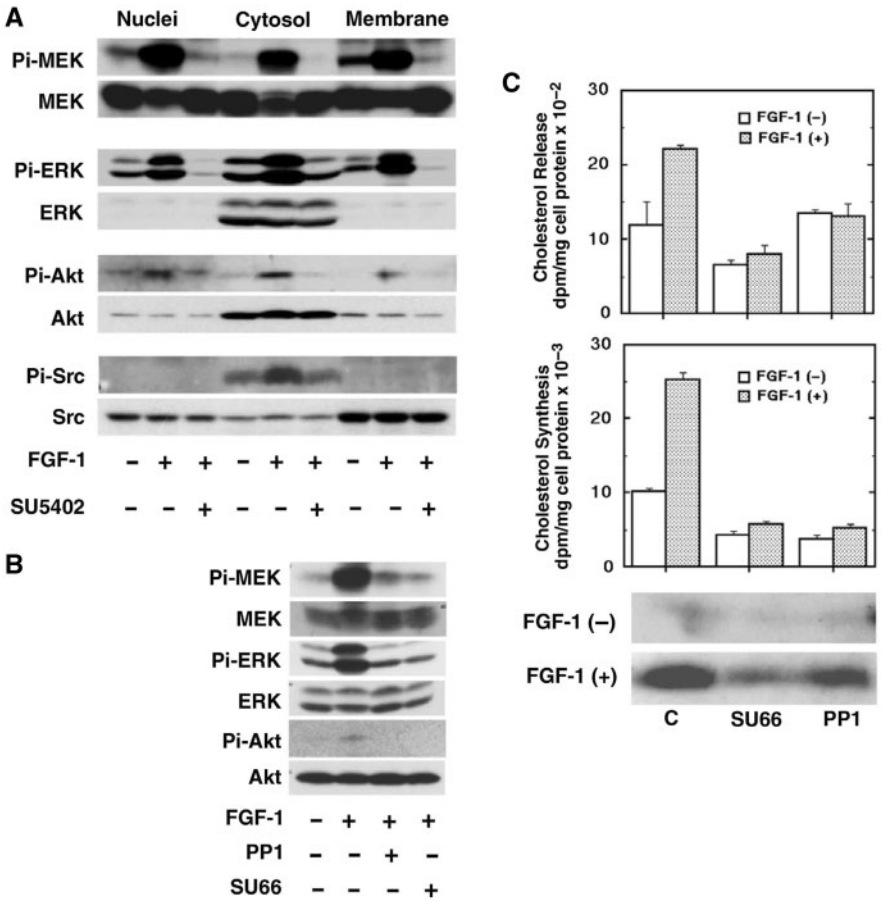

Fig. 1. **Involvement of the FGF-1 receptor and Src in the FGF-1-induced reactions in astrocytes.** (A) Rat astrocytes were pre-treated with the FGF-1 receptor inhibitor (SU5402, 10 μM) for 1 h, and stimulated with FGF-1 (50 ng/ml) for 5min. Cytosol and membrane fractions were prepared, and protein of each fraction was analysed by western blotting for MEK, ERK, Akt and Src proteins and their phosphorylated form (Pi). (B) The cells were pre-treated with Src inhibitors (PP1 or SU6656, 10 μM) for 1 h and stimulated by FGF-1 (50 ng/ml) for 5min. Cell protein was analysed by western blotting for the signal proteins and their phosphorylated form (Pi). (C) Cellular cholesterol release was measured in the presence of FGF-1 (50 ng/ml), SU6656 (10 μM) or PP1 (10 μM) (Top). Cholesterol biosynthesis was determined upon incubation with SU6656 (10 μM) or PP1 (10 μM) for 1 h and then with FGF-1 (0 or 50 ng/ml) for 5 h (Middle). ApoE secretion was measured under stimulation with FGF-1 (0 or 100 ng/ml) for 24 h in the presence of SU6656 (5 μM) or PP1 (5 μM). The conditioned medium was analysed by western blotting for apoE.

damage of CNS, and seems to play a role in regeneration of nerve cells and healing of the injury (*6–15*). Therefore, it is important to understand the background of molecular mechanism for this reaction and the recovery process of the brain damage. We discovered that apoE-HDL production is stimulated by FGF-1 in astrocytes by an autocrine mechanism and helps healing of the brain cryo-injury (*16–19*). FGF-1 up-regulates apoE-HDL biogenesis by using at least three independent signalling pathways, a PI3K/Akt pathway for transport/secretion of apoE, an MEK/ERK pathway for cholesterol and lipid biosynthesis, and an independent pathway for apoE transcription (*19*). All of these pathways are probably initiated by the interaction of FGF-1 with its receptors (*19*, *20*).

We here characterized the involvement of Src in these FGF-1-induced signalling pathways. The results are summarized as follows (Fig. 5): (i) FGF-1-induced phosphorylation of MEK, ERK, Akt and Src proteins and these phosphorylations were inhibited by an inhibitor of the FGF-1 receptor. (ii) FGF-1-induced phosphorylation of MEK, ERK and were all inhibited by Src inhibitors and by siRNA of Src, and FGF-1-induced cholesterol synthesis, cholesterol release and apoE secretion were inhibited by Src inhibitors. (iii) Induction by FGF-1 of the genes related to lipid metabolism was not inhibited either by Src inhibitors or by Src siRNA. We concluded that FGF-1 induces MEK, ERK and Akt phosphorylation and cholesterol synthesis, cholesterol release and apoE secretion being mediated by the Src kinase. In this FGF-1-induced signalling pathway, Src seems located downstream of the FGFR1 and upstream of the MEK/ERK and PI3K pathways. Furthermore, these data showed that induction of the genes related to lipid metabolism such as apoE and HMG-CoA reductase is independent of the Src pathway.

We recently demonstrated that induction of apoE gene by FGF-1 is under the dual control, by the MEK/ERK

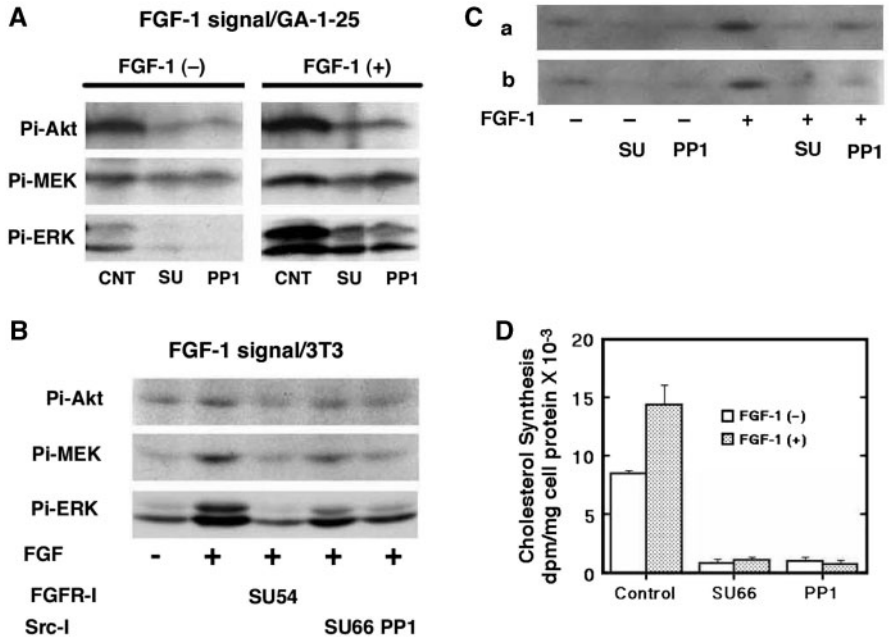

Fig. 2. **Involvement of the FGF-1 receptor and Src in the FGF-1-induced reactions in astrocytes in rat astrocytoma cells and mouse fibroblast.** (A) Inhibition of the FGF-1-induced reactions by Src inhibitors in GA-1/25 cells. The cells were stimulated with FGF-1 (50 ng/ml) for 5 min after pre-treatment with the Src inhibitors (PP1 or SU6656, 10 μM) for 1 h. The cell protein was analysed by western blotting for phosphorylated forms of MEK, ERK and Akt (Pi). (B) Inhibition of the FGF-1-induced reactions by the inhibitors of the FGF-1 receptor or Src in 3T3-L1 cells. The cells were stimulated with FGF-1 (50 ng/ml) for 5 min after pre-treatment with SU5402, PP1 or SU6656, 10 μM, for 1 h. Cell protein was analysed by western blotting for phosphorylated forms of MEK, ERK and Akt (Pi). (C) Inhibition of the FGF-1-induced secretion of apoE by Src inhibitors in GA-1/25 cells. After 16 h blank incubation, the cells were stimulated with FGF-1 (0 or 50 ng/ml) for 24 h in the presence SU6656 (5 μM) or PP1 (5 μM), and further incubated for 16 h (a). The cells were incubated with FGF-1 (0 or 50 ng/ml) for 24 h, then with SU6656 (5 μM) or PP1 (5 μM) for 16 h, and further incubated for 16 h (b). The conditioned medium was analysed by western blotting for apoE. (D) Inhibition of the FGF-1-induced cholesterol synthesis by Src inhibitors. GA-1/25 cells were incubated with SU6656 (10 μM) or PP1 (10 μM) for 1 h and then with FGF-1 (0 or 50 ng/ml) for 5 h. Cholesterol synthesis was determined as described in the text.

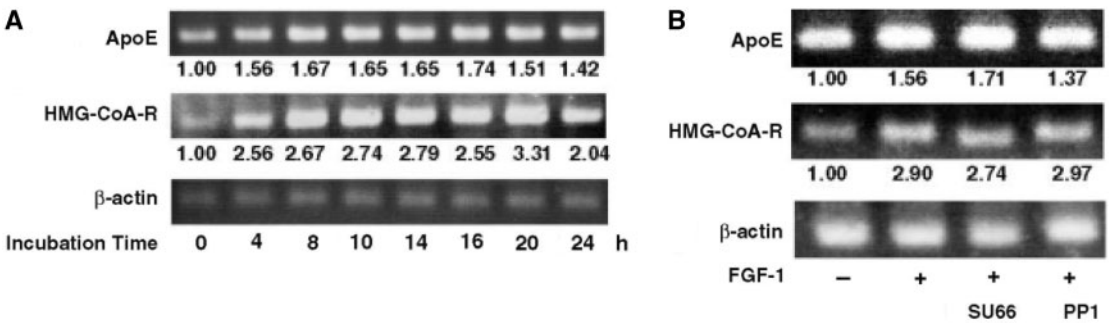

Fig. 3. **Expression of mRNA of the lipid metabolism-related genes induced by FGF-1 in rat astrocytes.** (A) mRNA expression of the lipid-related genes in a time course. Rat astrocytes were stimulated with 50 ng/ml of FGF-1 for the time indicated. Total RNA was extracted and subjected to reverse transcription and cDNA amplification for apoE, HMG-CoA reductase and β-actin. (B) Effect of Src inhibitors on mRNA expression of the lipid-related genes. Rat astrocytes were stimulated with 50 ng/ml of FGF-1 for 8 h after pre-treatment with Src inhibitors (PP1, SU6656) for 1 h. Total RNA was extracted and subjected to reverse transcription and cDNA amplification for apoE, HMG-CoA reductase and β-actin. Each band was digitally scanned by using an EPSON GT-X700 and Adobe Photoshop software. Numbers below each band indicates relative increase of its intensity from the controls after standardized for β-actin.

pathway to induce synthesis of cholesterol and 25-OH-cholesterol to activate LXRα and by induction of the LXRα gene through an unknown signalling pathway (*20*). The present results provided somewhat confusing information for understanding the differential signalling network for induction of biogenesis of apoE-HDL by FGF-1 in astrocytes. (i) Although the increase of cholesterol biogenesis is mediated by the MEK/ERK pathway (*19*) and it is suppressed by inhibiting Src, induction of HMG-CoA reductase by FGF-1 seems independent of this

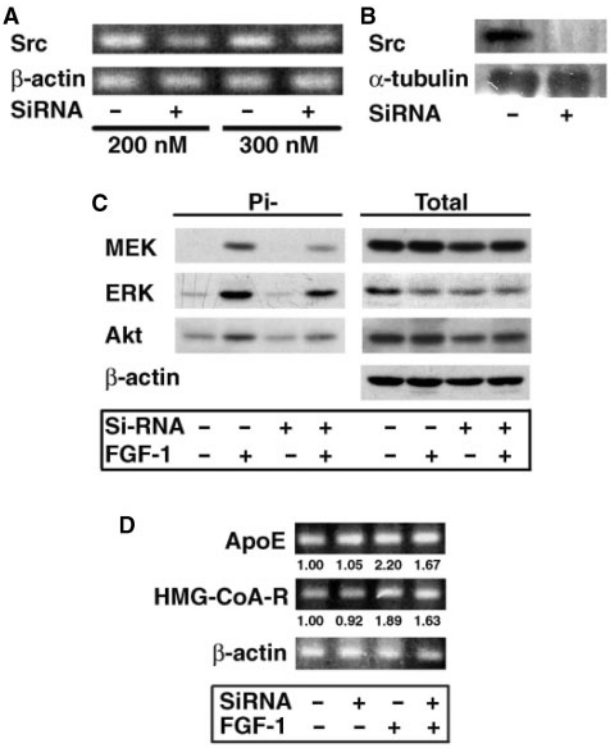

Fig. 4. **Effect of the treatment with Src siRNA.** (A) Effect of siRNA on expression of Src. Rat astrocytes were incubated with fresh Opti-mem for 10 h and then incubated with Src RNAi at 200 nM and 300 nM for 24 h. Total RNA was extracted and subjected to reverse transcription and cDNA amplification by using primer pairs for Src as described in the text. (B) Effect of Src siRNA on expression of Src protein. Rat astrocytes were incubated with fresh Opti-mem for 10 h and then incubated with 200 nM Src RNAi for 24 h. The cytosol protein was analysed by western blotting for α-tublin protein. (C) Rat astrocytes were pre-treated with 200 nM Src siRNA as above. After 5 min stimulation by FGF-1, cell protein was analysed by western blotting for MEK, ERK, Akt and their phosphorylated form (Pi). (D) Rat astrocytes were pre-treated with 200 nM Src RNAi as above. The cells were incubated with FGF-1 for 8 h and total RNA was subjected to reverse transcription and cDNA amplification by using primer pairs for apoE, HMG-CoA reductase and β-actin. Each band was digitally scanned by using an EPSON GT-X700 and Adobe Photoshop software. Numbers below each band indicates relative increase of its intensity from the controls after standardized for β-actin.

pathway indicating that cholesterol biosynthesis is stimulated by FGF-1 at an other step(s) than HMG-CoA reductase as well. (ii) Although expression of the apoE gene is induced by FGF-1 through production of cholesterol and 25-OH-cholesterol via the MEK/ERK pathway (*20*), LXRα induction directly increases the apoE gene transcription independently of this pathway (*19*). This view is consistent with the previous suggestion that enhancement of cholesterol biosynthesis by FGF-1 is due to the demand by stimulation of cell growth (*25*). Induction of the apoE gene transcription by FGF-1 may not all necessarily be associated with cholesterol biosynthesis and shown to be partially independent of either PI3K/Akt or MEK/ERK pathways as mentioned earlier (*19*).

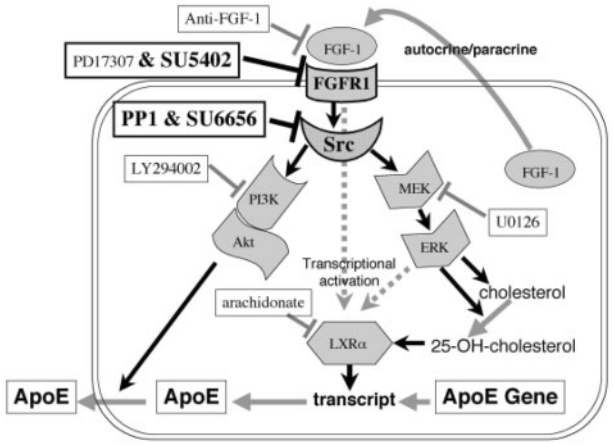

Fig. 5. **Schematic diagram for signalling pathways for FGF-1 to stimulate production of apoE-HDL in rat astrocytes.** FGF-1 is released by astrocytes in the lesions of brain damage and stimulates those cells in autocrine/paracrine fashion (*16–18*). This stimulation is mediated by FGFR1, and subsequently uses the MEK/ERK pathway to increase the synthesis of cholesterol and 25-OH-cholesterol, activate LXRα and enhance transcription of the apoE gene, and the PI3K/Akt pathway to increase apoE-HDL secretion (*19*, *20*). FGF-1 also enhances production of LXRα being independent of the aforementioned pathways (*19*, *20*). Src was shown to mediate signals from FGFR1 to the MEK/ERK and P13K/Akt pathways.

FUNDING

This study was supported by International HDL Award Program, in part by grants-in-aid from The Ministries of Education, Science, Technology, Culture and Sports, and of Health, Welfare and Labour of Japan, and by the Program for Promotion of Fundamental Studies in Health Sciences of National Institute of Biomedical Innovation.

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Ito, J. and Yokoyama, S. (2004) *Non-neural Cells of the Nervous System: Function and Dysfunction* (Hertz, L., ed.), pp. 519–534, Elsevier, Amsterdam
2. Pitas, R.E., Boyles, J.K., Lee, S.H., Foss, D., and Mahley, R.W. (1987) Astrocytes synthesize apolipoprotein E and metabolize apolipoprotein E-containing lipoproteins. *Biochim. Biophys. Acta* **917**, 148–161
3. Nakai, M., Kawamata, T., Taniguchi, T., Maeda, K., and Tanaka, C. (1996) Expression of apolipoprotein E mRNA in rat microglia. *Neurosci. Lett.* **211**, 41–44
4. Weiler-Guttler, H., Sommerfeldt, M., Papandrikopoulou, A., Mischek, U., Bonitz, D., Frey, A., Grupe, M., Scheerer, J., and Gassen, H.G. (1990) Synthesis of apolipoprotein A-I in pig brain microvascular endothelial cells. *J. Neurochem.* **54**, 444–450
5. Mockel, B., Zinke, H., Flach, R., Weis, B., Weiler-Guttler, H., and Gassen, H.G. (1994) Expression of apolipoprotein A-I in porcine brain endothelium in vitro. *J. Neurochem.* **62**, 788–798

6. Boyles, J.K., Pitas, R.E., Wilson, E., Mahley, R.W., and Taylor, J.M. (1985) Apolipoprotein E associated with astrocytic glia of the central nervous system and with nonmyelinating glia of the peripheral nervous system. *J. Clin. Invest.* **76**, 1501–1513
7. Muller, H.W., Gebicke-Harter, P.J., Hangen, D.H., and Shooter, E.M. (1985) A specific-37,000-dalton protein that accumulates in regenerating but not in nonregenerating mammalian nerves. *Science* **228**, 499–501
8. Dawson, P.A., Schechter, N., and Williams, D.L. (1986) Induction of rat E and chicken A-I apolipoproteins and mRNAs during optic nerve degeneration. *J. Biol. Chem.* **261**, 5681–5684
9. Ignatius, M.J., Gebicke-Harter, P.J., Skene, J.H.P., Schilling, J.W., Weisgraber, K.H., Mahley, R.W., and Shooter, E.M. (1986) Expression of apolipoprotein E during nerve degeneration and regeneration. *Proc. Natl Acad. Sci. USA* **83**, 1125–1129
10. Snipes, G.J., McGuire, C.B., Norden, J.J., and Freeman, J.A. (1986) Nerve injury stimulates the secretion of apolipoprotein E by nonneuronal cells. *Proc. Natl Acad. Sci. USA* **83**, 1130–1134
11. Mahley, R.W. (1988) Apolipoprotein E: Cholesterol transport protein with expanding role in cell biology. *Science* **240**, 622–630
12. Harel, A., Fainaru, M., Shafer, Z., Hernandez, M., Cohen, A., and Schwartz, M. (1989) Optic nerve regeneration in adult fish and apolipoprotein A-I. *J. Neurochem.* **52**, 1218–1228
13. Graham, D.I., Horsburgh, K., Nicoll, J.A., and Trasdale, G.M. (1999) Apolipoprotein E and the response of the brain to injury. *Acta Neurochir. Suppl.* **73**, 89–92
14. Haasdijk, E.D., Vlug, A., Mulder, M.T., and Jaarsma, D. (2002) Increased apolipoprotein E expression correlates with the onset of neuronal degeneration in the spinal cord of G93A-SOD1 mice. *Neurosci. Lett.* **335**, 29–33
15. Aoki, K., Uchihara, T., Sanjo, N., Nakamura, A., Ikeda, K., Tsuchiya, K., and Wakayama, Y. (2003) Increased expression of neuronal apolipoprotein E in human brain with cerebral infarction. *Stroke* **34**, 875–880
16. Tada, T., Ito, J., Asai, M., and Yokoyama, S. (2004) Fibroblast growth factor 1 is produced prior to apolipoprotein E in the astrocytes after cryo-injury of mouse brain. *Neurochem. Int.* **45**, 23–30
17. Ueno, S., Ito, J., Nagayasu, Y., Furukawa, T., and Yokoyama, S. (2002) An acidic fibroblast growth factor-like factor secreted into the brain cell culture medium upregulates apoE synthesis, HDL secretion and cholesterol metabolism in rat astrocytes. *Biochim. Biophys. Acta* **1589**, 261–272
18. Ito, J., Nagayasu, Y., Lu, R., Kheirollah, A., Hayashi, M., and Yokoyama, S. (2005) Astrocytes produce and secrete FGF-1, which promotes the production of apoE-HDL in a manner of autocrine action. *J. Lipid Res.* **46**, 679–686
19. Ito, J., Nagayasu, Y., Okumura-Noji, K., Lu, R., Nishida, T., Miura, Y., Asai, K., Kheirollah, A., Nakaya, S., and Yokoyama, S. (2007) Mechanism for FGF-1 to regulate biogenesis of apolipoprotein E-high density lipoprotein in astrocytes. *J. Lipid Res.* **48**, 2020–2027
20. Lu, R., Ito, J., Iwamoto, N., Nishimaki-Mogami, T., and Yokoyama, S. (2009) Fibroblast growth factor-1 induces expression of LXRa and production of 25-hydroxycholesterol to up-regulate apolipoprotein E gene transcription in rat astrocytes. *J. Lipid Res.* **50**, 1156–1164
21. Kilkenny, D.M., Rocheleau, J.V., Price, J., Reich, M.B., and Miller, G.G. (2003) c-Src regulation of fibroblast growth factor-induced proliferation in murine embryonic fibroblasts. *J. Biol. Chem.* **278**, 17448–17454
22. Ito, J., Zhang, L., Asai, M., and Yokoyama, S. (1999) Differential generation of high-density lipoprotein by endogenous and exogenous apolipoproteins in cultured fetal rat astrocytes. *J. Neurochem.* **72**, 2362–2369
23. Zhang, L., Ito, J., Kato, T., and Yokoyama, S. (2000) Cholesterol homeostasis in rat astrocytoma cells GA-1. *J. Biochem.* **128**, 837–845
24. Hashimoto, M., Sagara, Y., Langford, D., Everall, I.P., Mallory, M., Everson, A., Digicaylioglu, M., and Masliah, E. (2002) Fibroblast growth factor 1 regulates signaling via the glycogen synthase kinase-3beta pathway. Implications for neuroprotection. *J. Biol. Chem.* **277**, 32985–32991
25. LaVallee, T.M., Prudovsky, I.A., McMahon, G.A., Hu, X., and Maciag, T. (1998) Activation of the MAP kinase pathway by FGF-1 correlates with cell proliferation induction while activation of the Src pathway correlates with migration. *J. Cell Biol.* **141**, 1647–1658