日本財団 助成事業
The Nippon Foundation

２００２年度日中医学協会共同研究等助成事業報告書

－在留中国人研究者研究助成－

2003 年 3 月 15 日

財団法人 日中医学協会
理事長 殿

研究者氏名 張 仲行 (張仲行 印)
所属機関名 大阪大学 大学院 医学系研究科
指導責任者氏名 松澤 佑次
職名 教授
所在地 〒565-0871 大阪府 吹田市 山田丘 2-2
電話 06-6879-3732 内線 3732

1．研究テーマ

弱粥状動脈硬化の成因に関する分子生物学的研究

2．本年度の研究業績

(1) 学会・研究会等における発表 (有) ・ 無 (学会名・演題)

学会名
The 67th Annual Scientific Meeting of the Japanese Circulation Society March 28-30, 2003, Fukuoka

演題
Decreased Cholesterol Efflux from Werner Syndrome Fibroblasts and Its Phenotypic Correction by the Adenovirus-Mediated Gene Delivery of Wild-Type Cdc42

(2) 学会誌等に発表した論文 有 ・ (無) (雑誌名・論文名)

一日中医学協会助成事業一

Werner症候群におけるコレステロール引き抜き及び細胞内脂質輸送に関する研究

Werner Syndrome Fibroblasts Have Defective Cholesterol Efflux and Its Correction by the Introduction of Cdc42, a Member of Rho-GTPases Families

研究者氏名	張　仲衍
中国所属機関	天津第一中心病院心臓内科
日本研究機関	大阪大学大学院医学系研究科
指導責任者	松澤　佑次
共同研究者	平野賢一、山下静也、松澤佑次

Summary

Cholesterol efflux from the cells is the initial and crucial step of reverse cholesterol transport, a major protective system against atherosclerosis. Werner syndrome (WS) is characterized by the early onset of senescent phenotypes including premature atherosclerotic cardiovascular disease and cancer, though the underlying molecular mechanism is not fully understood yet. In the present study, we examined cholesterol efflux as well as intracellular lipid trafficking in the WS fibroblasts. We found that cholesterol efflux mediated by apolipoprotein AI and high density lipoprotein was markedly reduced. The intracellular lipid trafficking of fluorescence ceramide was also markedly retarded in the living WS fibroblasts, demonstrated by fluorescence recovery after photobleaching. To know the mechanism for the above abnormal lipid transport, we examined the expression of levels of ATP-binding cassette transporter-1 (ABCA1) and Cdc42, a member of RhoGTPases families. Although the protein levels of ABCA1 were not altered, those of Cdc42 were markedly reduced in the WS fibroblasts. The introduction of WRN cDNA increased cholesterol efflux along with the induction of Cdc42 in the WS cells. The introduction of wild-type Cdc42 completely corrected both of the retarded intracellular lipid transport and decreased cholesterol efflux in the WS cells. These effects were observed in TD cells as well. In conclusion, WS fibroblasts had defective cholesterol efflux and intracellular lipid transport in association with the decreased expression of Cdc42. The roles of Cdc42 on lipid transport appear independent of ABCA1.

Key words: aging, atherosclerosis, Cdc42, cholesterol efflux, fluorescence recovery after photobleaching, reverse cholesterol transport, vesicular transport, Werner's syndrome.

Introduction

Atherosclerotic cardiovascular disease is a major cause of death in the well-developed countries. Reverse cholesterol transport (RCT) is one of the major protective systems against atherosclerosis, in which high density lipoprotein (HDL) particles or free apolipoprotein (Apo) AI removes cholesterol from lipid-laden cells and delivers it to the liver, a terminal of RCT. We have continued to elucidate the significance of RCT by analyzing the pathophysiology of disorders with abnormal HDL metabolism including cholesteryl ester transfer protein deficiency, Tangier disease (TD), and familial HDL deficiency (FHD).

Cholesterol efflux from the cells is the initial and crucial step of RCT. In 1999 the ATP-binding cassette transporter-1 (ABCA1) gene was found mutated in patients with TD, which is a familial HDL deficiency characterized by the defective cholesterol efflux as well as the retarded intracellular lipid transport in their cells. Many researchers including ours have tried to elucidate the detailed molecular mechanism for cholesterol efflux, because the enhancement of cholesterol efflux is believed to be one of the novel strategies for the development of treatment against atherosclerosis.

The gain and loss of function studies demonstrated that ABCA1 is prerequisite for apo AI-mediated cholesterol efflux from the cells. In addition to the identification of novel ABCA1 mutations in the Japanese patients with TD and FHD, we found that the expression of Cdc42, a member of Rho-GTPases families, was markedly decreased in the TD cells by using the cDNA subtraction techniques and subsequently demonstrated that MDCK cells expressing dominant negative form of Cdc42 had reduced cholesterol efflux. Furthermore, we reported that ABCA1 may interact with Cdc42 in the over-expressing cells and that the ABCA1-induced changes in the actin cytoskeletons might be inhibited by the introduction of dominant negative form of Cdc42. More recently, we reported that Cdc42 was decreased in *in vitro* and *in vivo* aged human fibroblasts in association with retarded intracellular lipid kinetics around the Golgi apparatus. We proposed a hypothesis that Cdc42 might be important for intracellular lipid trafficking and its export from the cells.

Werner syndrome (WS), which was first described by Dr. Otto Werner at the Kiel University in 1904, is characterized by the presence of premature aging, atherosclerosis and malignancy. Many patients with WS died of acute myocardial infarction and cancer at their 40s. Since it was found that the WS is caused by mutations at the WRN locus on chromosome 8p in 1996, the characteristics of WRN protein have been

analyzed to show that WRN protein has activities for DNA helicase, ATPase, and exonuclease. However, molecular mechanism underlying the premature atheroscleosis is still not fully understood yet.

In the present study, we examined the cellular lipid transport in the WS fibroblasts. We found that the WS fibroblasts had defective cholesterol efflux and retarded intracellular lipid trafficking along with the decreased expression of Cdc42. Although these cellular characteristics in the WS cells were similar to those in TD fibroblasts, the expression of ABCA1 protein was not apparently changed. We demonstrated that the transfection of WRN cDNA corrected the abnormal lipid transport and, furthermore, the introduction of Cdc42 completely corrected the observed abnormal lipid transport in the WS cells. Although the etiologies were different between WS and TD, these data indicated the possible relationship between Cdc42 and lipid transport.

Materials and Methods

-Antibodies-

To detect WRN protein, rabbit anti-sera against the carboxy-terminal of WRN protein was kindly provided from Dr. Oshima (Seattle, Washington). Polyclonal antibody was Anti-c-myc antibody (9E10) and anti-Cdc42 antibody were purchased from Santa Cruz Biotechnology. The antibody against ABCA1 was recently generated in our laboratory.

-Cells and tissue culture-

WS1 (RCB0155), WS2 (RCB0387), and W-V (RCB0252) were obtained from the RIKEN cell bank (Tsukuba, Japan). WS1 and WS2 were passage skin fibroblasts from unrelated female patients with WS. W-V was a SV40-transformed fibroblasts from a male patient with WS. TD was a fibroblast cell line from a female patient with TD who was homozygous for the R1851X truncated mutation in the ABCA1 gene, as recently reported. The immunoreactive mass of WRN protein were not detectable in either of the WS cells used, by the antibody against the caroboxy-terminus of WRN protein. Normal human fibroblasts were obtained from 2 4-, 48-, and 54-year-old female after the informed consent, as described previously. The fibroblasts were passaged by 1: 4 split to increase cell population doubling level (PDL) by 2 on passage. The cells were cultured according to the standard conditions in minimum essential medium (MEM) supplemented with L-glutamine, nonessential amino acids, and 10 % of fetal calf serum in a humidified 5 % CO2 atomosphere at 37 ℃.

-Plasmids and transfection-

A plasmid encoding full-length WRN cDNA driven by CMV promoter, pBSCA-6xHis WRN, was kindly provided from Dr. Junko Oshima (Washington University, Seattle). cDNA of myc- tagged wild-type Cdc42 (myc-Cdc42-WT) was kindly provided from Drs. Kenji Takaishi and Yoshimi Takai (Department of Molecular Biology and Biochemistry , Osaka University, Japan). Plasmid DNAs were transfected into the SV40-transformed WRN fibroblasts by using Lipofectamine 2000.

Western blot analysis

Fibroblasts were lysed and passed 10 times through 25-gause needles. Samples were run on SDS-PAGE, blotted onto polyvinylidene difluoride (PVDF) membranes (Millipore). For the analysis of ABCA1 protein, the extraction buffer containing higher concentrations of urea was used, as reported previously. The detection was performed with enhanced chemiluminescence (ECL) plus kit (Amersham) according to the manufacturer's protocol.

Construction of Adenovirus Vectors and their Expression in Fibroblasts

Adenovirus vector encoding myc-Cdc42-WT or LacZ was constructed according to the protocol of the Adeno-X expression system (Clontech). The titer of the virus stock was assessed by a plaque formation assay using HEK293 cells and expressed as plaque formation units. The infection with adenovirus was carried out by incubating cells in serum-free medium for 1 hour at 37 ℃ under gentle agitation. The medium was exchanged with a complete medium and the cells were further incubated at CO2 incubator. Five days after infection with the indicated m.o.i., the cells were applied to the experiments.

Fluorescence recovery after photobleaching (FRAP)

Fluorescence recovery after photobleaching (FRAP) experiment was carried out as follows; Cells were stained with C6-NBD-Ceramide (Molecular Probes) for 30 min at 4 ℃. After washed twice with ice cold PBS, cells were incubated with complete media at 37 ℃ for 30 min. A beam of light using 488 nm laser lines was focused on the indicated part of C6-NBD-Ceramide positive regions in the living cells by a confocal laser microscopy (Zeiss LSM 510, Carl Zeiss Co., Ltd.). Typically, 30 iterations were required for complete or

almost complete photobleaching. After the appropriate bleach pulse, FRAP was monitored at the bleached area until 30 seconds after bleaching. The relative fluorescence was measured by dividing the fluorescence in the FRAP area by that in the reference spot. The recovery was reasonably fit by a single exponential function. After the values of relative fluorescence were plotted, the time constant was calculated.

-Cholesterol Efflux Assay-

Cellular cholesterol efflux was assayed by a modification of a published method. Briefly, the release of radioactive cholesterol to a given acceptor (*e.g.* 10 μg/mL of human apo AI or HDL) was measured by scintillation counting of filtered aliquots of acceptor-containing medium and expressed as the fraction of the total isopropanol-soluble label in the cells plus the label release to the medium. All efflux assays were done in triplicate. The cells were pre-labeled with 3H-cholesterol by incubation for 18 hours at 37 ℃ in a CO2 incubator with MEM supplemented with 20 % fetal calf serum (FCS) and an acyl CoA: cholesterol acyltransferase (ACAT) inhibitor (F-1394, Fuji Rebio, Japan). In all experiments, fractional efflux was corrected for the small amount of radioactivity released to MEM without an acceptor present.

Results

Marked Reduction of Apo AI- and HDL-mediated Cholesterol Efflux in Fibroblasts from Werner Syndrome

We tested apolipoprotein (apo) AI- and HDL3-mediated cholesterol efflux in the passaged skin fibroblasts from two unrelated patients with WS, as shown in **Figures 1-A** and **1-B**. Apo AI-mediated cholesterol efflux was markedly reduced in the two WS fibroblasts lines by ~70 %, comparing with normal fibroblasts (n=3) (**Figure 1-A**). The observed reduction in cholesterol efflux was not so severe in the WS cells as in the fibroblasts from a patient with Tangier disease (TD). HDL3-mediated cholesterol efflux was also decreased in the WS cells, which was comparable to that in TD cells (**Figure 1-B**). We also examined the cholesterol efflux from the SV40-immortalized WS and normal fibroblasts. We could obtain the similar data in the immortalized cells, showing the reduction in both apo AI- and HDL3-mediated cholesterol efflux in the WS cells (data not shown).

-Markedly Retarded Intracellular Lipid Kinetics in WS Fibroblasts-

Next, we examined the intracellular lipid trafficking in the living WS cells by the fluorescence recovery after photobleaching technique (FRAP), using a fluorescent ceramide (C6-NBD-ceramide, Molecular Probe) as a probe. FRAP is the powerful tool to examine the intracellular transport of protein as well as lipids in the living cells. The ceramide used in the present study is believed to be metabolized and accumulated in the Golgi apparatus and to be sorted and transported to the plasma membrane via vesicular transport. Furthermore, the previous studies showed that the kinetics of this ceramide closely reflects that of cholesterol. We analyzed the intracellular lipid transport around the Golgi apparatus using the fluorescent ceramide. After incubation, C6-NBD-ceramide accumulated around the Golgi apparatus. After bleaching, the recovery of fluorescence intensity was monitored and time constants were measured in the defined region (**Figure 2**). We found that the time constants for recovery were significantly prolonged in the WS fibroblasts compared with those of controls (18.5 ± 2.5 seconds versus 7.2 ± 2.1 seconds, respectively ($p < 0.01$)). These data indicated that the intracellular transport of lipids may be markedly retarded in the WS cells.

-Expression levels of ABCA1 and Cdc42-

These data indicated that the WS cells had the defective cholesterol efflux and retarded intracellular lipid trafficking, which resembled those of TD cells. In order to know the mechanism underlying the abnormal lipid transport in the WS cells, we first analyzed the expression levels of ABCA1 mRNA and protein, which is known to be a prerequisite molecule for apo AI-mediated cholesterol efflux and the cause of TD. RNase protection assay revealed that the expression levels of ABCA1 mRNA was not altered in the WS cells (data not shown). As shown in **Figure 3**, the immunoreactive mass of ABCA1 protein was comparable to those of normal cells. There could be a possibility that intracellular localization and activity of ABCA1 might be affected in the WS cells. However, because HDL3-mediated cholesterol efflux, which is believed a very complex process, was also decreased in the WS cells, we focused on molecules other than ABCA1. We tested the expression levels of scavenger receptor class B type I (SR-BI), which is known to facilitate HDL3-mediated cholesterol efflux, showing no expression in either normal or WS fibroblasts. Next, we analyzed the expression of levels of Cdc42, which is a member of RhoGTPases families. This is because we have reported that MDCK cells stably expressing dominant negative form of Cdc42 had defective cholesterol efflux, proposing a hypothesis that this G protein may be important for lipid transport. As shown in **Figures 3**, the expression levels of Cdc42 protein were markedly reduced in the WS fibroblasts , comparing with fibroblasts from normal subjects.

-ransfection of WRN cDNA Increased Cholesterol Efflux and the Expression of Cdc42 in WS cells

In order to correlate the above findings to WRN protein, we examined the effect of transfection of WRN

cDNA on cholesterol effux and the expression levels of ABCA1 and Cdc42. As shown in **Figure 4**, the introduction of WRN cDNA induced a significant increase in both apo AI- and HDL-mediated cholesterol efflux along with the increase of Cdc42, whereas the expression levels of ABCA1 were not changed by the transfection of WRN cDNA.

-Introduction of Wild-type Cdc42 Completely Corrected Abnormal Lipid Transport-

Finally, to correlate the decreased expression of Cdc42 with the defective cholesterol efflux as well as retarded intracellular lipid transport, we examined the effect of introduction of wild-type Cdc42 into the WS cells as well as the TD cells. The adenovirus-mediated introduction of wild-type Cdc42 into the WS cells was successful (data not shown). **Figure 5** shows the effect of wild-type Cdc42 on the intracellular lipid transport determined by FRAP. The introduction of WT-Cdc42 completely corrected the abnormal intracellular kinetics of cearmide in the WS cells as well as TD cells. We also tested the effect on apo AI- and HDL-mediated cholesterol efflux in the normal as well as WS fibroblasts (**Figure 6**). We could observe the dose-dependent increase in cholesterol efflux in the WS cells. In addition, in the normal fibroblasts, the cholesterol efflux was significantly increased. These data indicated that the observed abnormalities in lipid transport could be corrected by WT-Cdc42 without the introduction of WRN protein. Furthermore, we tested the effect of wild-type Cdc42 on apo AI- and HDL-mediated cholesterol efflux in the TD cells (**Figure. 7**), showing that Cdc42 completely corrected the cholesterol efflux. These data suggested that Cdc42-mediated lipid transport is independent of ABCA1.

Discussion

The present study for the first time demonstrated the marked reduction of apo AI- and HDL-mediated cholesterol efflux and retardation of intracellular lipid transport in the WS fibroblasts along with the decreased expression of Cdc42. We demonstrated that the introduction of WRN cDNA completely rescued the observed phenotypes including the abnormal expression of Cdc42. Finally, we also found that the complementation of wild-type Cdc42 completely corrected the above abnormal lipid transport without the introduction of WRN.

Recently, we have proposed that Cdc42, a member of Rho-GTPases families, may be important for cellular lipid transport, since we found the decreased expression of this type of small G protein in cells from TD cells. In the present study, we detected the comparable expression of ABCA1 transporter in the WS cells, however the cholesterol efflux was markedly reduced. Even though there could be possibilities that the activity or intracellular localization of ABCA1 might be affected, we think that the present findings indicated that ABCA1 is pre-requisite, but may not be sufficient for cholesterol efflux. Furthermore, the introduction of WT-Cdc42 completely corrected the abnormalities in cholesterol efflux and intracellular lipid transport without changes in either the expression levels or cell surface expression of ABCA1 protein (data not shown). The effect of introduction of Cdc42-WT on cholesterol efflux was also observed in the TD cells lacking ABCA1 transporter (Hirano K, et al. Unpublished observation). These data indicated that the effect of Cdc42 on cholesterol efflux might be independent of ABCA1, though it needs to be clarified how Cdc42 mediates lipid transport.

It would be of importance to know whether or not the macrophages from patients with WS or WRN knockout mice may have defective cholesterol efflux, which we did not have chance to test. Saito and his colleagues reported that some patients with WS suffered from tendon xanthomatosis and their macrophages had higher cholesterol content. Our unpublished data showed that cholesterol content was higher in WS fibroblasts than normal cells, which was associated with the increased expression of caveolin-1 and decrease in LDL receptor (Zhang, et al. Unpublished). It still remains to be investigated the mechanism for the reduction of Cdc42 protein in the WS fibroblasts. There have been revealed that WRN DNA helicase protein has multi-function to regulate recombination, transcription, and apoptosis, though the introduction of WRN cDNA into the cells induced the expression of Cdc42. Further studies are required to know the regulatory mechanism.

Even though the molecular characteristics of WRN protein as DNA helicase have been extensively clarified, there are still many points needed to be clarified including molecular mechanism for premature aging. In addition to the present study, we have reported that the expression of Cdc42 was decreased in *in vitro* and *in vivo* aged human fibroblasts in association with the retarded intracellular lipid transport. Because Cdc42 is know to play various essential cell biological activities including regulation of actin dynamics, cell cycle, transformation, vesicular transport, and lipid transport, the malfunction of this type of small G protein could induce the many abnormalities at the individual as well as cellular levels. The finding in this report might provide important implications for the expression of premature atherosclerosis and other senescent phenotypes in patients with WS.

References

1. Gray, M. D., Wang, L., Youssoufian, H., Martin, G. M., Oshima, J. (1998) *Exp. Cell. Res.* **242**, 487-494.
Tsukamoto, K., Hirano, K., Yamashita, S., Sakai, N., Ikegami, C., Zhang, Z., Matsuura, F., Hiraoka, H., Matsuyama, A., Ishigami, M., Matsuzawa. (2002) *Arterioscler. Thromb. Vasc. Biol.* **22**, 1899-1904.

2. Glomset, J.A. (1968) *J. Lipid Res.* **9**, 155-67.
Tall, A.R. (1998) *Eur. Heart J.* Suppl A, A31-5.

3. Yamashita, S., Hirano, K., Sakai, N., Matsuzawa, Y. (2000) *Biochim. Biophys. Acta.* **1529**, 257-275.
Hirano, K., Yamashita, S., Matsuzawa, Y. (2000) *Curr. Opin. Lipidol.* **11**, 589-596.

4. Brooks-Wilson, A., Marcil, M., Clee, S.M., Zhang, L., Roomp, K., van Dam, M., Yu, L., Brewer, C., Collins, A.J., Zwinderman, A.H., Molhuizen, H.O.F., Loubser, O., Ouelette, B.F.F., Fichter, K., Asbourne-Excoffon, K.J.D., Sensen, C.W., Scherer, S., Mott, S., Denis, M., Martindale, D., Frohlich, J., Morgan, K., Koop, B., Pimstone, S., Kastelein, J.J. P., Genest, J.Jr., Hayden, M.R. (1999) *Nat. Genet.* **22**, 336-345.

5. Bodzioch, M., Orso, E., Klucken, J., Langmann, T., Bottcher, A., Diederich, W., Drobnik, W., Barlage, S., Buchler, C., Porsch-Ozcurumez, M., Kaminski, W.E., Hahmann, H.W., Oette, K., Rothe, G., Aslandis, C., Lackner, K.J., Schmitz, G. (1999) *Nat. Genet.* **22**, 347-351.

6. Rust, S., Rosier, M., Funke, H., Real, J., Amoura, Z., Piette, J.C., Deleuze, J.F., Brewer, H.B., Duverger, N., Denefle, P., Assmann, G. (1999) *Nat. Genet.* **22**, 352-355.

7. Nishida, Y., Hirano, K., Tsukamoto, K., Nagano, M., Ikegami, C., Roomp, K., Ishihara, M., Sakane, N., Zhang, Z., Tsujii, K., Matsuyama, A., Ohama, T., Matsuura, F., Ishigami, M., Sakai, N., Hiraoka, H., Hattori, H., Wellington, C., Yoshida, Y., Misugi, S., Hayden, M.R., Egashira, T., Yamashita, S., Matsuzawa, Y. (2002) *Biochem Biophys Res Commun.* **290**: 713-721.

8. Hirano, K., Matsuura, F., Tsukamoto, K., Zhang, Z., Matsuyama, A., Takaishi, K., Komuro, R., Suehiro, T., Yamashita, S., Takai, Y., Matsuzawa, Y. (2000) *FEBS Lett.* 484, 275-279.

9. Tsukamoto, K., Hirano, K., Tsujii, K., Ikegami, C., Zhongyan, Z., Nishida, Y., Ohama, T., Matsuura, F., Yamashita, S., Matsuzawa, Y. (2001) *Biochem Biophys Res Commun.* **287**, 757-765.

10. Wellington, C.L., Walker, E.K.Y., Suarez, A., Kwok, A., Bissada, N., Singaraja, R., Yang, Y., James, E., Wilson, J. E., Francone, O., McManus, B.M., Hayden, M.R. (2002) *Lab Invest.* 82: 273-283.

11. Bortnick, A.E., Rothblat, G.H., Stoudt, G., Hoppe, K.L., Royer, L.J., McNeish, J., Francone, O.L. (2000) The correlation of ATP-binding cassette 1 mRNA levels with cholesterol efflux from various cell lines. *J Biol Chem.* 275: 28 634-28640.

12. Orso, E., Broccardo, C., Kaminski, W.E., Bottcher, A., Liebisch, G., Drobnik, W., Gotz, A., Chambenoit, O., Diederich, W., Langmann, T., Spruss, T., Luciani, M.F., Rothe, G., Lackner, K.J., Chimini, G., Schmitz, G. (2000) *Nat. Genet.* 24, 192-196.

13. Drobnik, W., Lindenthal, B., Lieser, B., Ritter, M., Christiansen Weber, T., Liebisch, G., Giesa, U., Igel, M., Borsukova, H., Buchler, C., Fung-Leung, W.P., Von Bergmann, K., Schmitz, G. (2001) ATP-binding cassette transporter A1 (ABCA1) affects total body sterol metabolism. *Gastroenterology*. 120: 1203-1211.

14. Kojima, K., Abe-Dohmae, S., Arakawa, R., Murakami, I., Suzumori, K., Yokoyama, S (2001). Progesterone inhibits apolipoprotein-mediated cellular lipid release: a putative mechanism for the decrease of high-density lipoprotein. Biochim Biophys Acta. 1532: 173-184.

15. Tanaka, A.R., Ikeda, Y., Abe-Dohmae, S., Arakawa, R., Sadanami, K., Kidera, A., Nakagawa, S., Nagase, T., Aoki, R., Kioka, N., Amachi, T., Yokoyama, S., Ueda, K. (2001) Human ABCA1 contains a large amino-terminal extracellular domain homologous to an epitope of Sjogren's Syndrome. *Biochem. Biophys. Res. Commun*. 283, 1019-25.

16. Hirano, K., Yamashita, S., Nakagawa, Y., Ohya, T., Matsuura, F., Tsukamoto, K., Okamoto, Y., Matsuyama, A., Matsumoto, K., Miyagawa, J., Matsuzawa, Y. (1999) *Circ Res.* **85**: 108-116.

17. Komuro, R., Yamashita, S., Sumitsuji, S., Hirano, K., Maruyama, T., Nishida, M., Matsuura, F., Matsuyama, A., Sugimoto, T., Ouchi, N., Sakai, N., Nakamura, T., Funahashi, T., Matsuzawa, Y. (2000) *Circulation.* 101: 2446-2448.

18. Ohama, T., Hirano, K., Zhang, Z., Aoki, R., Tsujii, K., Nakagawa-Toyama, Y., Tsukamoto, K., Ikegami, C., Matsuyama, A., Ishigami, M., Sakai, N., Hiraoka, H., Ueda, K., Yamashita, S., Matsuzawa, Y. (2002) *Biochem. Biophys. Res. Commun.* **296**, 625-630.

19. Oshima, J., Campisi, J., Tannock, T. C., Martin, G. M. (1995) *J. Cell. Physiol.* **162**, 277-283.

20. Yu, C. E., Oshima, J., Fu, Y. H., Wijsman, E. M., Hisama, F., Alisch, R., Matthews, S., Nakura, J., Miki, T., Ouais, S., Martin, G. M., Mulligan, J., Schellenberg, G. D. (1996) *Science.* **272**, 258-262.

21. Ye, L., Miki, T., Nakura, J., Oshima, J., Kamino, K., Rakugi, H., Ikegami, H., Higaki, J., Edland, S. D., Martin, G. M., Ogihara, T. (1997) *Am. J. Med. Genet.* **68**, 494-498.

22. Gray, M. D., Shen, J. C., Kamath-Loeb, A. S., Blank, A., Sopher, B. L., Martin, G. M., Oshima, J., Loeb, L. A. (1997) *Nat. Genet.* **17**, 100-103.

23. Balajee, A. S., Machwe, A., May, A., Gray, M. D., Oshima, J., Martin, G. M., Nehlin, J. O., Brosh, R., Orren, D. K., Bohr, V. A. (1999) *Mol. Biol. Cell.* **10**, 2655-2668.

24. Moser, M. J., Kamath-Loeb, A. S., Jacob, J. E., Bennett, S. E., Oshima, J., Monnat, R. J. Jr. (2000) *Nucleic. Acids. Res.* **28**, 648-654.

25. Oshima, J. (2000) *Bioessays.* **22**, 894-901.

26. Leventhal, A. R., Chen, W., Tall, A. R., Tabas, I. (2001) J. Biol. Chem. 276, 44976-44983.

27. Chen, W., Sun, Y., Welch, C., Gorelik, A., Leventhal, A. R., Tabas, I., Tall, A. R. (2001) J. Biol. Chem. 276, 43564-43569.

28. Mori, S., Yokote, K., Morisaki, N., Saito, Y., Yoshida, S. (1990) Eur. J. Clin. Invest. 20: 137-142.

29. Saito, Y. (1991) Gerontology. 37 Suppl 1: 48-53.

30. Murano, S., Nakazawa, A., Saito, I., Masuda, M., Morisaki, N., Akikusa, B., Tsuboyama, T., Saito, Y. (1997) 43 Suppl 1: 43-52.

Figure 1 Decreased cholesterol efflux in WS fibroblasts

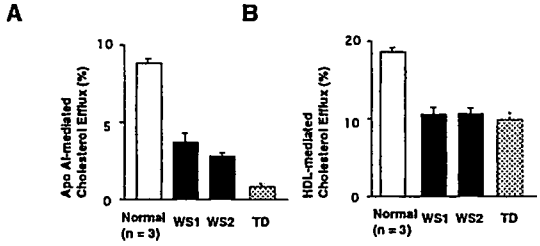

Figure 2 Markedly Retarded Intracellular Lipid Kinetics in WS Fibroblasts

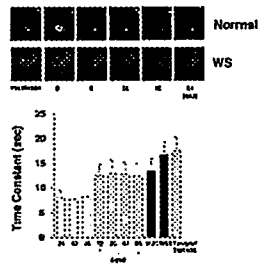

Figure 3 Expression levels of ABCA1 were not altered but the levels of Cdc42 protein were markedly reduced in the WS fibroblasts

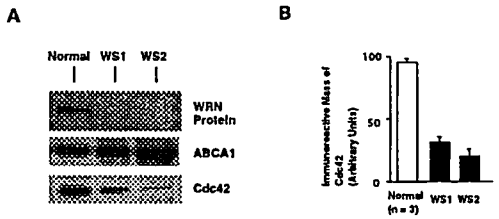

Figure 4 Transfection of WRN cDNA Increased Cholesterol Efflux and the Expression of Cdc42 in WS cells

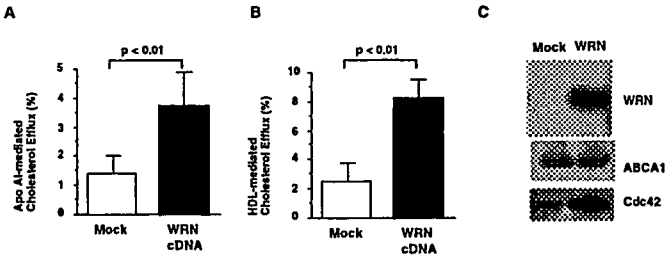

Figure 5 The effect of wild-type Cdc42 on the intracellular lipid transport determined by FRAP

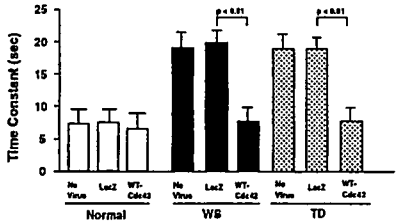

Figure 6 The introduction of WT-Cdc42 completely corrected the decreased cholesterol efflux in the WS cells.

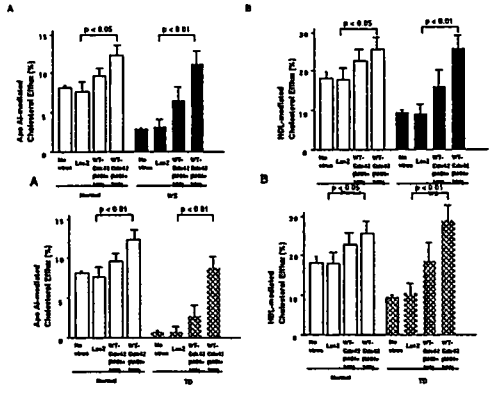

Figure 7 The introduction of WT-Cdc42 completely corrected the decreased cholesterol efflux in the TD cells.