



Fibronectin-containing High-Density Lipoprotein is Associated with Cancer Cell Adhesion and Proliferation

Hisamatsu, Eriko ; Nagao, Manabu ; Toh, Ryuji ; Irino, Yasuhiro ; Iino, Takuya ; Hara, Tetsuya ; Tanaka, Hidekazu ; Satomi-Kobayashi, Seimi ;...

(Citation)

The Kobe journal of the medical sciences, 66(1):40-48

(Issue Date)

2020

(Resource Type)

departmental bulletin paper

(Version)

Version of Record

(JaLCD0I)

<https://doi.org/10.24546/81012553>

(URL)

<https://hdl.handle.net/20.500.14094/81012553>



Fibronectin-containing High-Density Lipoprotein is Associated with Cancer Cell Adhesion and Proliferation

ERIKO HISAMATSU^{1*}, MANABU NAGAO^{2*}, RYUJI TOH^{2#},
YASUHIRO IRINO³, TAKUYA IINO³, TETSUYA HARA¹, HIDEKAZU TANAKA¹,
SEIMI SATOMI-KOBAYASHI¹, TATSURO ISHIDA¹ and KEN-ICHI HIRATA^{1,2}

**These two co-first authors contributed equally to this work.*

#Corresponding author.

¹*Division of Cardiovascular Medicine, Kobe University Graduate School of Medicine, Kobe, Japan*

²*Division of Evidence-based Laboratory Medicine, Kobe University Graduate School of Medicine, Kobe, Japan*

³*Central Research Laboratories, Sysmex Corporation, Kobe, Japan*

Received 17 February 2020 / Accepted 10 April 2020

Key words: High-density lipoprotein, Fibronectin, Focal Adhesion Kinase, Cancer progression.

A large amount of evidence suggests that high-density lipoprotein (HDL) has anti-atherosclerotic properties. HDL-cholesterol (HDL-C) has also been widely used as a marker of cardiovascular disease. Recently, it was reported that plasma HDL-C levels are inversely correlated with cancer risk. However, the relationship between HDL and cancer pathophysiology remains unknown.

Here, we sought to investigate the effect of HDL on cancer progression. First, we focused on fibronectin—an essential extracellular matrix glycoprotein—as an HDL-associated protein and found that only 7.4% of subjects in this study had fibronectin in HDL isolated from their plasma. The fibronectin-containing HDL (FN-HDL) increased the phosphorylation of focal adhesion kinase (FAK) in HeLa cells compared to HDL without fibronectin, further inducing the phosphorylation in a dose-dependent manner. Second, we found that fibronectin-treated HDL activated the phosphorylation of FAK, and its upstream effector blocked the phosphorylation induced by FN-HDL. Finally, we demonstrated that FN-HDL promoted cancer cell proliferation and adhesion compared to HDL without fibronectin. Our study showed the possible mechanism by which FN-HDL enhanced cancer cell proliferation and adhesion via the FAK signaling pathway. Further investigation of the roles of HDL components in tumorigenesis might provide novel insight into cancer pathophysiology.

A large amount of evidence has demonstrated that high-density lipoprotein (HDL) has anti-atherosclerotic properties and high-density lipoprotein cholesterol concentration (HDL-C) inversely correlates with the risk for cardiovascular disease (1-3). The athero-protective effect of HDL is due to its key role in reverse cholesterol transport (RCT) (4), whereas it is well known that in response to cardiovascular disease, HDL has anti-inflammatory, anti-oxidative, anti-apoptotic, and anti-thrombotic effects and vasodilatory properties independent of RCT (5, 6). In fact, HDL has a cardioprotective effect beyond its athero-protective properties, leading to reduction in the incidence of heart failure in humans (7, 8). These beneficial effects of HDL largely depend on the multiple components of HDL including paraoxonase-1 (PON1), platelet-activating factor acetylhydrolase (PAF-AH), and sphingosine-1-phosphate (S1P) (9). In contrast, several studies have indicated a negative impact of HDL components on atherosclerosis. For instance, serum amyloid A (SAA) and myeloperoxidase (MPO), carried on HDL, generate dysfunctional HDL, leading to the increased risk of coronary artery disease (CAD) (10-13). Given that HDL functions as a carrier of various enzymes and molecules, it is likely that circulating HDL is not only associated with cardiovascular disease but also influences the development of systemic diseases represented by cancer.

Cancer is the second leading cause of death globally. Accumulating evidence suggests that dyslipidemia caused by excessive consumption of Western-style diet, followed by obesity, was involved in tumorigenesis of various cancers (14, 15). Accordingly, it has been reported that abnormal metabolism of lipoproteins is associated with different types of cancers (16, 17). Regarding HDL, some studies indicate a negative correlation between blood HDL-C level and cancer incidence (18-20). Yang, et al. reported that 1 mg/dL reduction in circulating HDL-C was related to a 14% increased risk of cancer prevalence (21).

Alwaili, et al. identified 67 HDL-associated proteins in patients with CAD, using shotgun proteomics (22). Among those proteins, fibronectin, an essential extracellular matrix glycoprotein, was found only in HDL isolated

from the subjects with acute coronary syndrome (22). Fibronectin participates in multiple stages of cancer progression, namely cancer growth, migration, and invasion (23-26). Mitra, et al. found that fibronectin upregulated the c-Met/FAK/Src signaling pathway by binding to cellular $\alpha_5\beta_1$ -integrin in ovarian cancer cells (26) and suggested that fibronectin promotes ovarian cancer invasion and metastasis. However, how HDL with fibronectin affects cancer progression is still unknown. Here, we sought to elucidate the biological mechanism of action of fibronectin-containing HDL (FN-HDL) in the process of tumorigenesis.

MATERIALS AND METHODS

Clinical participants and HDL preparation

The Kobe Cardiovascular Marker Investigation (CMI) registry is a single-center registry of patients referred to Kobe University Hospital with cardiovascular disease, which is conducted to identify blood-based biomarkers that have utility in predicting cardiovascular disease. The study protocol was in accordance with the ethical guidelines of the 1975 Declaration of Helsinki. The study was approved by the Ethics Review Committee at Kobe University (Japan) and was registered at the UMIN Clinical Trials Registry (identification number 000030297). Written informed consent was obtained from all patients prior to enrollment in the study. We randomly enrolled 27 patients with stable angina who did not have a medical history of cancer in the past 5 years, from CMI registry for the present study and isolated HDL from their plasma as previously reported (27). In brief, HDL fraction was isolated by ultracentrifugation, and its purity was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and subsequent Coomassie staining.

Cell culture

HeLa cells were purchased from the European Collection of Authenticated Cell Cultures (Porton Down, UK) and cultured in Dulbecco's modified Eagle's medium (DMEM) (WAKO, Tokyo, Japan) supplemented with 10% fetal bovine serum (FBS) and 100 IU/ml penicillin/streptomycin (P/S) (each from Sigma-Aldrich, St. Louis, MO, USA) at 37 °C and 5% CO₂.

Treatment of HDL with fibronectin

To test whether HDL isolated from the subjects contains fibronectin, we performed western blot analysis as described below. We incubated Non-FN-HDL (100 µg/ml) with fibronectin (10 µg/ml) overnight and then used that for stimulation of HeLa cells.

Western blotting

Western blotting was performed as previously reported (28). In brief, cells were washed twice with cold phosphate-buffered saline (PBS) (-) and harvested with ice-cold lysis buffer (20-mM HEPES (pH 7.4), 1% NP40, 1% SDS, and 150-mM NaCl). Proteins from the lysates were separated using SDS-PAGE and transferred onto polyvinylidene difluoride membranes (Thermo Fisher, Tokyo, Japan). After blocking with 5% milk for 1 h, the membranes were incubated with primary antibodies overnight at 4 °C and then with horseradish peroxidase-conjugated secondary antibodies for 1 h at room temperature. The signals were detected using the Amersham Imager 600 (GE Healthcare, Tokyo, Japan). The following antibodies were used in this study: anti-phosphor-FAK antibody (Thermo Fisher, #44-624G), anti-FAK antibody (BD, #610087), anti-Fibronectin antibody (Sigma Aldrich, #F0916), and anti-ApoA1 antibody (Santa Cruz, #sc-376818).

Cell proliferation assay

Three hundred cells were placed into a 60 mm dish and incubated in DMEM with 10% FBS and 1% P/S at 37 °C and 5% CO₂ overnight. Subsequently, cells were cultured with 10 µg/ml HDL or vehicle for 7 days, followed by crystal violet staining. Cells were washed twice with PBS (-) and fixed for 5 minutes with 4% paraformaldehyde. After fixation, cells were stained with 0.1% crystal violet.

Cell adhesion assay

Twenty thousand cells were placed into a Matrigel (CORNING, Tokyo, Japan) coated 96 well plate and incubated in serum-free medium. Afterwards, the cells were treated with 10 µg/ml HDL and incubated for 20 min. After washing the cells 4 times with PBS (-), cell adhesion was assessed using a cell counting kit (CCK)-8 assay kit (Cell Counting Kit-8, DOJINDO, Kumamoto, Japan) according to manufacturer's protocol.

RESULTS

In order to replicate a study by Alwaili, *et al.* (22), we examined whether HDL contained fibronectin by western blotting. Interestingly, HDL from only 2 subjects, out of 27, contained fibronectin (Figure 1A). Although the sample size was too small to draw any definite conclusions, the comparisons of patient profiles and conventional lipid profiles between patients with and without fibronectin in their HDL particles are shown in Table I. The FN-HDL showed greater phosphorylation of focal adhesion kinase (FAK) compared to HDL without fibronectin (Non-FN-HDL) in HeLa cells (Figure 1B). FAK is known to be a non-receptor protein tyrosine kinase and contributes to cancer progression and metastasis by activating cell proliferation, migration, and adhesion (29).

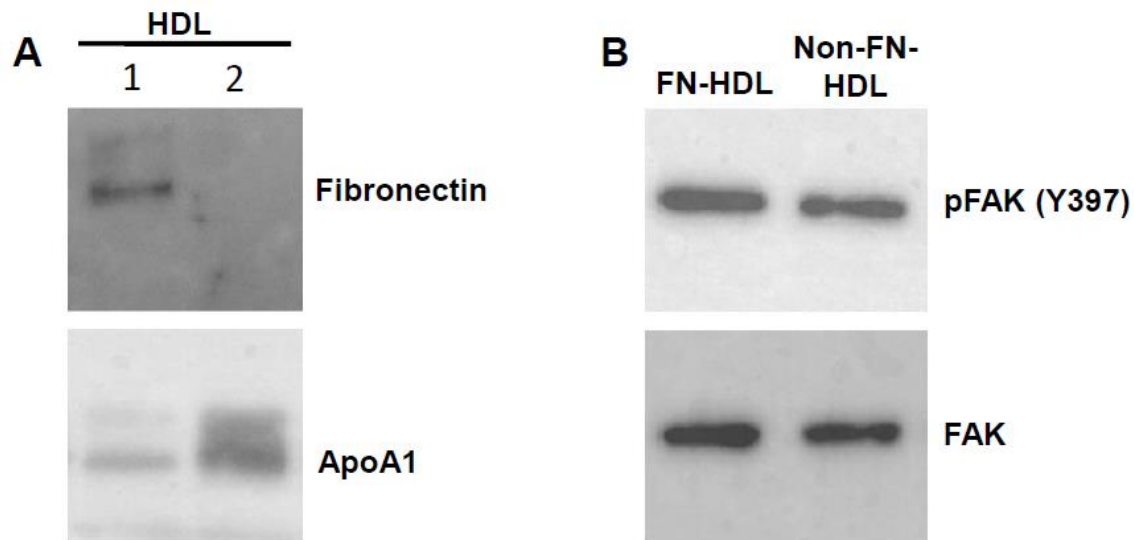


Figure 1. Fibronectin-containing HDL (FN-HDL) highly enhances the phosphorylation of focal adhesion kinase (FAK).

A) Fibronectin contained in HDL was detected by western blot analysis with anti-Fibronectin antibody. B) HeLa cells were incubated with 10 μ g/ml HDL, which either contained fibronectin (FN-HDL) or not (Non-FN-HDL). Afterwards, phosphor-FAK was evaluated by western blotting.

To investigate whether the FAK phosphorylation depends on the presence of fibronectin on HDL, we stimulated HeLa cells with fibronectin-treated HDL and showed that it increased the phosphor-FAK (Figure 2A). On the other hand, we confirmed that there was no effect on the FAK phosphorylation when fibronectin alone was used for the stimulation to the cells (Figure 2B). Additionally, FN-HDL upregulated the phosphorylation of FAK; however, it was inhibited by a calcium chelator ethylene glycol tetra-acetic acid (EGTA), which is known to inactivate integrin, an upstream effector in FAK signaling (Figure 2C).

To test the dose dependency of FN-HDL on the phosphorylation of FAK, we stimulated HeLa cells with FN-HDL and found that FN-HDL treatment upregulated the phosphorylation of FAK in a dose dependent manner (Figure 3A). On the other hand, treatment with ApoA1, a main protein moiety in HDL, did not enhance the phosphorylation of FAK (Figure 3A).

Next, we examined the effects of FN-HDL on cancer cell properties. FN-HDL activated the proliferation in HeLa cells better than Non-FN-HDL (4A). Moreover, FN-HDL also upregulated the adhesion of HeLa cells as compared to Non-FN-HDL (4B). These findings suggested that FN-HDL promoted cancer cell proliferation and adhesion via the FAK signaling pathway.

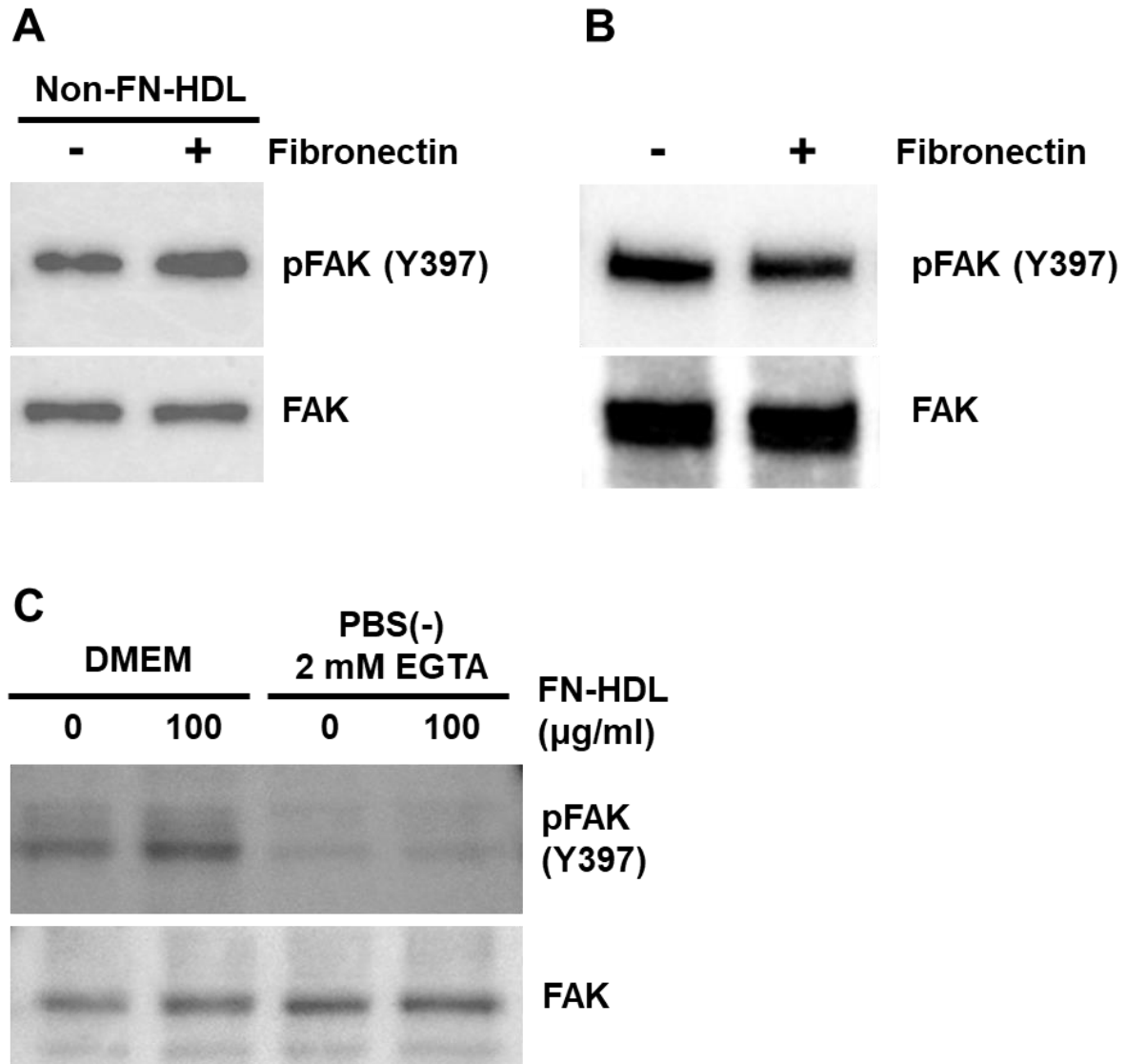


Figure 2. Phosphorylation of FAK due to FN-HDL is blocked by its upstream inhibitor.

A) Non-FN-HDL (100 μg/ml) was incubated with 10 μg/ml fibronectin. Subsequently, HeLa cells were incubated with the fibronectin-treated HDL (+) or non-treated HDL (-), followed by western blotting with antibodies as indicated. B) HeLa cells were incubated with the 10 μg/ml fibronectin alone, followed by western blotting using same antibodies as Figure 2A. C) HeLa cells were cultured in Dulbecco's modified Eagle's medium (DMEM) with FN-HDL or phosphate-buffered saline (PBS) (-) containing 2 mM ethylene glycol tetra-acetic acid (EGTA), a calcium chelator. Subsequently, western blot analysis was performed using antibodies as indicated.

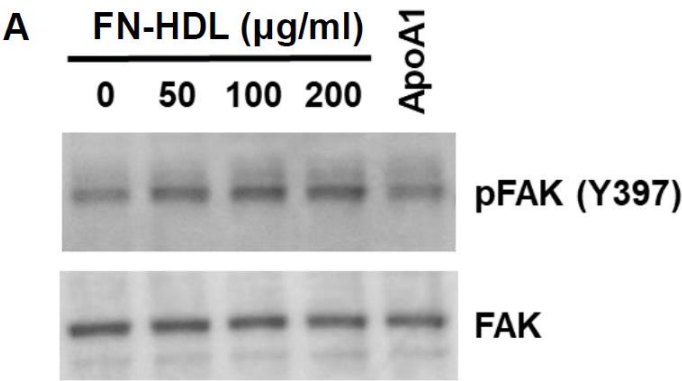


Figure 3.

HDL increases phosphor-FAK in a dose dependent manner.

A) HeLa cells were stimulated with recombinant ApoA1 (200 μg/ml) or FN-HDL (0, 50, 100, and 200 μg/ml). Phosphorylation of FAK was evaluated by western blotting.

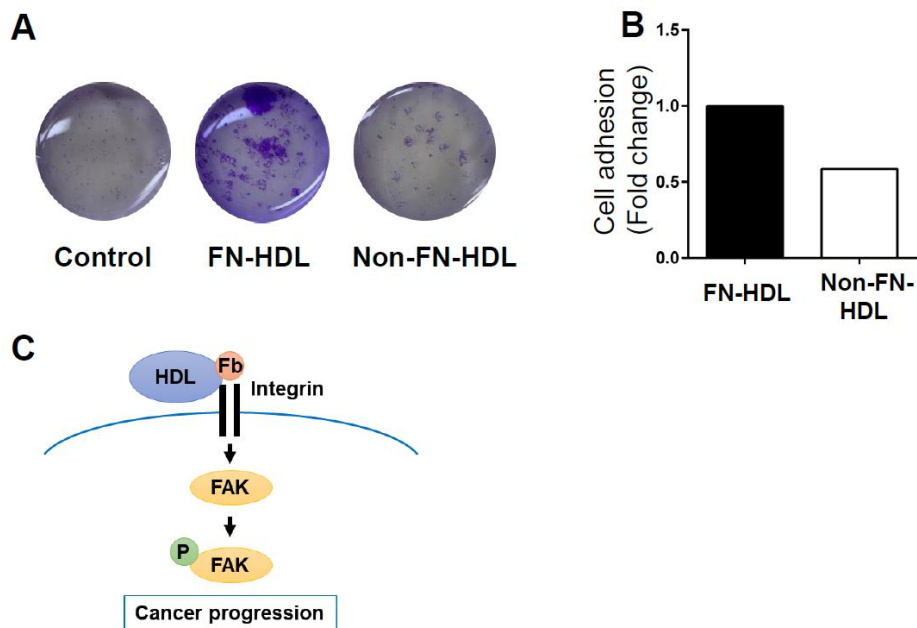


Figure 4. FN-HDL promoted cancer cell proliferation and adhesion.

A) Three hundred HeLa cells were placed into a 60mm dish and cultured with FN-HDL or Non-FN-HDL for 7 days, followed by crystal violet staining. B) HeLa cells were seeded into 96 well plate coated by Matrigel. Twenty minutes after incubation, cells were washed 4 times with PBS (-). Afterwards, cells adhered on the plate was quantified by Cell Counting Kit-8 (CCK-8) assay. C) A scheme showing the possible mechanism by which FN-HDL activates FAK signaling pathway.

Table I. Patient Characteristics

	FN-HDL (n = 2)	Non-FN-HDL (n = 25)
Age (years)	53±7	68.3±11.8
Gender (Male %)	100.0	72.0
BMI (kg/m ²)	26.7±0.75	25.2±3.1
Current smoking (%)	50.0	24.0
Hypertension (%)	100.0	80.0
Diabetes mellitus (%)	0.0	36.0
Dyslipidemia (%)	100.0	88.0
HbA1c (%)	5.95±0.15	6.3±1.2
Glucose (mg/dL)	106.5±3.5	106.3±30.2
Total cholesterol (mg/dL)	169.5±6.5	159.7±41.0
LDL-cholesterol (mg/dL)	104.0±12.0	89.6±33.3
HDL-cholesterol (mg/dL)	45.0±6.0	46.4±12.1
Triglyceride (mg/dL)	133.5±1.5	140.5±87.8
Apolipoprotein AI (mg/dL)	127.5±11.5	128.8±28.4
hs-CRP (mg/dL)	0.045±0.025	0.260±0.473

FN-HDL; Fibronectin-containing HDL, Non-FN-HDL; HDL without fibronectin, BMI; body mass index, LDL; low density lipoprotein, HDL; high density lipoprotein, hs-CRP; high sensitivity C-reactive protein.

DISCUSSION

Here, we evaluated the effect of FN-HDL on the pathological mechanisms of cancer progression. We found that HDL isolated from only 7.4% of subjects contained fibronectin, and FN-HDL increased the phospho-FAK levels, promoting cancer cell adhesion and proliferation (Figure 4C).

Low HDL-C level is related to the incidence of several cancers such as breast, prostate, and lung cancers (30-32). One of the possible reasons why low HDL-C correlates with the increasing risk of cancers is thought to be the contribution of Apolipoprotein A-I (ApoA-I), the major protein constituent of HDL. Many studies reported that ApoA-I suppressed cancer development and progression both *in vitro* and *in vivo* (33). Su, et al. revealed that ApoA-I and ApoA-I mimetic peptides inhibit tumor development in a mouse model of ovarian cancer by removing lysophosphatidic acid, a bioactive lysophospholipid which induces cell migration and invasion in ovarian cancer (34). Zamanian-Daryoush, et al. demonstrated that ApoA-I suppressed tumor growth and metastasis in multiple animal tumor models (35). In their study, a transgenic mouse model overexpressing the human apoA1 presented increased infiltration of M1 macrophages, known as an anti-tumor phenotype, and decreased tumor burden and metastasis. In contrast, apoA1-deficient mice showed highly enhanced tumor growth and reduced survival (35). On the other hand, several studies revealed the negative impact of HDL on cancer progression. Hayat, et al. demonstrated that presence of SAA on HDL altered HDL binding to the cell surface, possibly leading to cancer cell proliferation owing to the change in HDL-mediated cholesterol delivery to cells (36). Similar to the results for SAA, our data indicated that fibronectin on HDL mediated cancer cell proliferation and adhesion. Taken together, the results suggest that the pro- or anti-tumorigenic effects of HDL are highly likely to be attributed to the proteins carried by HDL.

Our study revealed that FN-HDL upregulated the phosphorylation of FAK in HeLa cells, a cervical cancer cell line. Fibronectin is overexpressed in many tumors and associated with survival and metastasis in patients with cancer (23-26). Zhou, et al. reported that fibronectin was activated in patients with cervical cancer, and its higher expression was associated with a poor prognosis for patients with cervical cancer (37). Moreover, numerous studies revealed the association between FAK and various cancers and the biological mechanisms by which FAK promotes cancer development and progression (38). FAK plays an important role in cell signaling systems and is highly expressed and phosphorylated in several cancers, including lung, pancreatic, gastric and ovarian cancers (39-42). Considering that fibronectin is one of the inducers of the FAK signaling pathway (26), it seems to be convincing that FN-HDL activates this pathway. Although there is not enough evidence that demonstrates involvement of HDL or HDL components in cancer pathophysiology, investigating the association between characteristics of HDL constituents and tumorigenesis might have significant implications in cancer treatment.

There are some limitations to our study. First, although we used HDL isolated from CAD patients who did not have a medical history of cancer in the past 5 years, we could not follow the incidence of cancer in these patients prospectively. Second, only 7.4% of the patients had fibronectin in their HDL; further elucidation of the pathological role of FN-HDL, using more patient samples is needed. Finally, we could not assess the concentration of fibronectin contained in FN-HDL and clarify why fibronectin on HDL activated the FAK signaling pathway, not the circulating fibronectin which is not carried by HDL (Figure 2A, B). There might be several mechanisms by which HDL affects the affinity of fibronectin to its receptors or the tissue transitivity of that.

In summary, we identified fibronectin in HDL particles, further demonstrating that FN-HDL promoted cancer cell proliferation and adhesion through the FAK signaling pathway. In the field of cardiovascular disease, several studies show the adverse role of dysfunctional HDL on atherogenesis and heart disease. Correspondingly, HDL has positive and negative effects on tumorigenesis and may work as a mediator of systemic diseases. Elucidation of the detailed mechanism by which HDL and its components regulate cancer development and progression might help develop a novel strategy in cancer therapeutics.

ACKNOWLEDGEMENTS

The authors thank Emiko Yoshida for her contributions to technical assistance. This work was supported by a Grant-in-Aid for Scientific Research (C) 18K08073 and 19K08491 from the Ministry of Education, Culture, Sports, Science and Technology of Japan. The Division of Evidence-based Laboratory Medicine, Kobe University Graduate School of Medicine, was established by an endowment fund from the Sysmex Corporation, Japan.

REFERENCES

1. **Castelli, W.P., Garrison, R.J., Wilson, P.W., Abbott, R.D., Kalousdian, S., and Kannel, W.B.** 1986. Incidence of coronary heart disease and lipoprotein cholesterol levels. The Framingham Study. *JAMA*. **256**: 2835-2838.
2. **Assmann, G., Schulte, H., von Eckardstein, A., and Huang, Y.** 1996. High-density lipoprotein cholesterol as a predictor of coronary heart disease risk. The PROCAM experience and pathophysiological implications for reverse cholesterol transport. *Atherosclerosis*. **124 Suppl**: S11-20.
3. **Sharrett, A.R., Ballantyne, C.M., Coady, S.A., Heiss, G., Sorlie, P.D., Catellier, D., Patsch, W., and Atherosclerosis Risk in Communities Study, G.** 2001. Coronary heart disease prediction from lipoprotein

- cholesterol levels, triglycerides, lipoprotein(a), apolipoproteins A-I and B, and HDL density subfractions: The Atherosclerosis Risk in Communities (ARIC) Study. *Circulation*. **104**: 1108-1113.
4. **Cuchel, M., and Rader, D.J.** 2006. Macrophage reverse cholesterol transport: key to the regression of atherosclerosis? *Circulation*. **113**: 2548-2555.
5. **de Goma, E.M., deGoma, R.L., and Rader, D.J.** 2008. Beyond high-density lipoprotein cholesterol levels evaluating high-density lipoprotein function as influenced by novel therapeutic approaches. *Journal of the American College of Cardiology*. **51**: 2199-2211.
6. **Nofer, J.R., Kehrel, B., Fobker, M., Levkau, B., Assmann, G., and von Eckardstein, A.** 2002. HDL and arteriosclerosis: beyond reverse cholesterol transport. *Atherosclerosis*. **161**: 1-16.
7. **Nagao, M., Toh, R., Irino, Y., Nakajima, H., Oshita, T., Tsuda, S., Hara, T., Shinohara, M., Ishida, T., and Hirata, K.I.** 2017. High-density lipoprotein protects cardiomyocytes from oxidative stress via the PI3K/mTOR signaling pathway. *FEBS open bio*. **7**: 1402-1409.
8. **Nagao, M., Nakajima, H., Toh, R., Hirata, K.I., and Ishida, T.** 2018. Cardioprotective Effects of High-Density Lipoprotein Beyond its Anti-Atherogenic Action. *Journal of atherosclerosis and thrombosis*. **25**: 985-993.
9. **Kosmas, C.E., Martinez, I., Sourlas, A., Bouza, K.V., Campos, F.N., Torres, V., Montan, P.D., and Guzman, E.** 2018. High-density lipoprotein (HDL) functionality and its relevance to atherosclerotic cardiovascular disease. *Drugs in context*. **7**: 212525.
10. **Coetzee, G.A., Strachan, A.F., van der Westhuyzen, D.R., Hoppe, H.C., Jeenah, M.S., and de Beer, F.C.** 1986. Serum amyloid A-containing human high density lipoprotein 3. Density, size, and apolipoprotein composition. *The Journal of biological chemistry*. **261**: 9644-9651.
11. **Prufer, N., Kleuser, B., and van der Giet, M.** 2015. The role of serum amyloid A and sphingosine-1-phosphate on high-density lipoprotein functionality. *Biological chemistry*. **396**: 573-583.
12. **Haraguchi, Y., Toh, R., Hasokawa, M., Nakajima, H., Honjo, T., Otsui, K., Mori, K., Miyamoto-Sasaki, M., Shinohara, M., Nishimura, K., Ishida, T., and Hirata, K.** 2014. Serum myeloperoxidase/paraoxonase 1 ratio as potential indicator of dysfunctional high-density lipoprotein and risk stratification in coronary artery disease. *Atherosclerosis*. **234**: 288-294.
13. **Tietge, U.J.F.** 2018. The impact of myeloperoxidase on HDL function in myocardial infarction. *Current opinion in endocrinology, diabetes, and obesity*. **25**: 137-142.
14. **Chandler, P.D., Song, Y., Lin, J., Zhang, S., Sesso, H.D., Mora, S., Giovannucci, E.L., Rexrode, K.E., Moorthy, M.V., Li, C., Ridker, P.M., Lee, I.M., Manson, J.E., Buring, J.E., and Wang, L.** 2016. Lipid biomarkers and long-term risk of cancer in the Women's Health Study. *The American journal of clinical nutrition*. **103**: 1397-1407.
15. **Agnoli, C., Grioni, S., Sieri, S., Sacerdote, C., Vineis, P., Tumino, R., Giurdanella, M.C., Pala, V., Mattiello, A., Chiodini, P., Iacoviello, L., De Curtis, A., Cattaneo, L., van Duijnhoven, F.J., Panico, S., and Krogh, V.** 2014. Colorectal cancer risk and dyslipidemia: a case-cohort study nested in an Italian multicentre cohort. *Cancer epidemiology*. **38**: 144-151.
16. **Lupien, L.E., Bloch, K., Dehairs, J., Traphagen, N.A., Feng, W.W., Dennis, T., Davis, W.L., Swinnen, J.V., Wells, W.A., Smits, N.C., Kuemmerle, N.B., Miller, T.W., and Kinlaw, W.B.** 2019. Endocytosis of very low-density lipoproteins: an unexpected mechanism for lipid acquisition by breast cancer cells. *Journal of lipid research*. **61**(2): 205-218.
17. **Allampallam, K., Dutt, D., Nair, C., Shetty, V., Mundle, S., Lisak, L., Andrews, C., Ahmed, B., Mazzone, L., Zorat, F., Borok, R., Muzammil, M., Gundroo, A., Ansaarie, I., and Raza, A.** 2000. The clinical and biologic significance of abnormal lipid profiles in patients with myelodysplastic syndromes. *Journal of hematotherapy & stem cell research*. **9**: 247-255.
18. **Pirro, M., Ricciuti, B., Rader, D.J., Catapano, A.L., Sahebkar, A., and Banach, M.** 2018. High density lipoprotein cholesterol and cancer: Marker or causative? *Progress in lipid research*. **71**: 54-69.
19. **Mondul, A.M., Weinstein, S.J., Virtamo, J., and Albanes, D.** 2011. Serum total and HDL cholesterol and risk of prostate cancer. *Cancer causes & control : CCC*. **22**: 1545-1552.
20. **van Duijnhoven, F.J., Bueno-De-Mesquita, H.B., Calligaro, M., Jenab, M., Pischon, T., Jansen, E.H., Frohlich, J., Ayyobi, A., Overvad, K., Toft-Petersen, A.P., Tjonneland, A., Hansen, L., Boutron-Ruault, M.C., Clavel-Chapelon, F., Cottet, V., Palli, D., Tagliabue, G., Panico, S., Tumino, R., Vineis, P., Kaaks, R., Teucher, B., Boeing, H., Drogan, D., Trichopoulou, A., Lagiou, P., Dilis, V., Peeters, P.H., Siersema, P.D., Rodriguez, L., Gonzalez, C.A., Molina-Montes, E., Dorronsoro, M., Tormo, M.J., Barricarte, A., Palmqvist, R., Hallmans, G., Khaw, K.T., Tsilidis, K.K., Crowe, F.L., Chajes, V., Fedirko, V., Rinaldi, S., Norat, T., and Riboli, E.** 2011. Blood lipid and lipoprotein concentrations and colorectal cancer risk in the European Prospective Investigation into Cancer and Nutrition. *Gut*. **60**: 1094-1102.
21. **Yang, C., Tian, G., Mi, J., Wei, X., Li, X., Li, X., Wang, W., and Wang, B.** 2015. Causal relevance of

- circulating high-density lipoprotein cholesterol with cancer: a Mendelian randomization meta-analysis. *Scientific reports*. **5**: 9495.
22. **Alwaili, K., Bailey, D., Awan, Z., Bailey, S.D., Ruel, I., Hafiane, A., Krimbou, L., Laboissiere, S., and Genest, J.** 2012. The HDL proteome in acute coronary syndromes shifts to an inflammatory profile. *Biochimica et biophysica acta*. **1821**: 405-415.
23. **Nam, J.M., Onodera, Y., Bissell, M.J., and Park, C.C.** 2010. Breast cancer cells in three-dimensional culture display an enhanced radioresponse after coordinate targeting of integrin $\alpha 5 \beta 1$ and fibronectin. *Cancer research*. **70**: 5238-5248.
24. **Kenny, H.A., Chiang, C.Y., White, E.A., Schryver, E.M., Habis, M., Romero, I.L., Ladanyi, A., Penicka, C.V., George, J., Matlin, K., Montag, A., Wroblewski, K., Yamada, S.D., Mazar, A.P., Bowtell, D., and Lengyel, E.** 2014. Mesothelial cells promote early ovarian cancer metastasis through fibronectin secretion. *The Journal of clinical investigation*. **124**: 4614-4628.
25. **Lou, X., Han, X., Jin, C., Tian, W., Yu, W., Ding, D., Cheng, L., Huang, B., Jiang, H., and Lin, B.** 2013. SOX2 targets fibronectin 1 to promote cell migration and invasion in ovarian cancer: new molecular leads for therapeutic intervention. *Omics : a journal of integrative biology*. **17**: 510-518.
26. **Mitra, A.K., Sawada, K., Tiwari, P., Mui, K., Gwin, K., and Lengyel, E.** 2011. Ligand-independent activation of c-Met by fibronectin and $\alpha 5 \beta 1$ -integrin regulates ovarian cancer invasion and metastasis. *Oncogene*. **30**: 1566-1576.
27. **Tsuda, S., Shinohara, M., Oshita, T., Nagao, M., Tanaka, N., Mori, T., Hara, T., Irino, Y., Toh, R., Ishida, T., and Hirata, K.I.** 2017. Novel mechanism of regulation of the 5-lipoxygenase/leukotriene B4 pathway by high-density lipoprotein in macrophages. *Scientific reports*. **7**: 12989.
28. **Nagao, M., Toh, R., Irino, Y., Mori, T., Nakajima, H., Hara, T., Honjo, T., Satomi-Kobayashi, S., Shinke, T., Tanaka, H., Ishida, T., and Hirata, K.** 2016. beta-Hydroxybutyrate elevation as a compensatory response against oxidative stress in cardiomyocytes. *Biochemical and biophysical research communications*. **475**: 322-328.
29. **Aboubakar Nana, F., Vanderputten, M., and Ocak, S.** 2019. Role of Focal Adhesion Kinase in Small-Cell Lung Cancer and Its Potential as a Therapeutic Target. *Cancers*. **11(11)**:1683.
30. **Furberg, A.S., Jasienska, G., Bjurstam, N., Torjesen, P.A., Emaus, A., Lipson, S.F., Ellison, P.T., and Thune, I.** 2005. Metabolic and hormonal profiles: HDL cholesterol as a plausible biomarker of breast cancer risk. The Norwegian EBBA Study. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology*. **14**: 33-40.
31. **Van Hemelrijck, M., Walldius, G., Jungner, I., Hammar, N., Garmo, H., Binda, E., Hayday, A., Lambe, M., and Holmberg, L.** 2011. Low levels of apolipoprotein A-I and HDL are associated with risk of prostate cancer in the Swedish AMORIS study. *Cancer causes & control : CCC*. **22**: 1011-1019.
32. **Kucharska-Newton, A.M., Rosamond, W.D., Schroeder, J.C., McNeill, A.M., Coresh, J., Folsom, A.R., and Members of the Atherosclerosis Risk in Communities, S.** 2008. HDL-cholesterol and the incidence of lung cancer in the Atherosclerosis Risk in Communities (ARIC) study. *Lung cancer*. **61**: 292-300.
33. **Georgila, K., Vyrla, D., and Drakos, E.** 2019. Apolipoprotein A-I (ApoA-I), Immunity, Inflammation and Cancer. *Cancers*. **11(8)**:1097.
34. **Su, F., Kozak, K.R., Imaizumi, S., Gao, F., Amneus, M.W., Grijalva, V., Ng, C., Wagner, A., Hough, G., Farias-Eisner, G., Anantharamaiah, G.M., Van Lenten, B.J., Navab, M., Fogelman, A.M., Reddy, S.T., and Farias-Eisner, R.** 2010. Apolipoprotein A-I (apoA-I) and apoA-I mimetic peptides inhibit tumor development in a mouse model of ovarian cancer. *Proceedings of the National Academy of Sciences of the United States of America*. **107**: 19997-20002.
35. **Zamanian-Daryoush, M., Lindner, D., Tallant, T.C., Wang, Z., Buffa, J., Klipfell, E., Parker, Y., Hatala, D., Parsons-Wingerter, P., Rayman, P., Yusufshaq, M.S., Fisher, E.A., Smith, J.D., Finke, J., DiDonato, J.A., and Hazen, S.L.** 2013. The cardioprotective protein apolipoprotein A1 promotes potent anti-tumorigenic effects. *The Journal of biological chemistry*. **288**: 21237-21252.
36. **Hayat, S., and Raynes, J.G.** 2000. Acute phase serum amyloid A protein increases high density lipoprotein binding to human peripheral blood mononuclear cells and an endothelial cell line. *Scandinavian journal of immunology*. **51**: 141-146.
37. **Zhou, Y., Shu, C., and Huang, Y.** 2019. Fibronectin promotes cervical cancer tumorigenesis through activating FAK signaling pathway. *Journal of cellular biochemistry*. **120(7)**
38. **Zhou, J., Yi, Q., and Tang, L.** 2019. The roles of nuclear focal adhesion kinase (FAK) on Cancer: a focused review. *Journal of experimental & clinical cancer research : CR*. **38**: 250.
39. **Ocak, S., Chen, H., Callison, C., Gonzalez, A.L., and Massion, P.P.** 2012. Expression of focal adhesion kinase in small-cell lung carcinoma. *Cancer*. **118**: 1293-1301.

40. **Parkin, A., Man, J., Timpson, P., and Pajic, M.** 2019. Targeting the complexity of Src signalling in the tumour microenvironment of pancreatic cancer: from mechanism to therapy. *The FEBS journal*. **286**: 3510-3539.
41. **Yin, K., Shang, M., Dang, S., Wang, L., Xia, Y., Cui, L., Fan, X., Qu, J., Chen, J., and Xu, Z.** 2018. Netrin1 induces the proliferation of gastric cancer cells via the ERK/MAPK signaling pathway and FAK activation. *Oncology reports*. **40**: 2325-2333.
42. **Siu, M.K.Y., Jiang, Y.X., Wang, J.J., Leung, T.H.Y., Han, C.Y., Tsang, B.K., Cheung, A.N.Y., Ngan, H.Y.S., and Chan, K.K.L.** 2019. Hexokinase 2 Regulates Ovarian Cancer Cell Migration, Invasion and Stemness via FAK/ERK1/2/MMP9/NANOG/SOX9 Signaling Cascades. *Cancers*. **11(6)**:813.