The Role of C1q as the Ligand of Discoidin Domain Receptor 2

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The Role of C1q as the Ligand of Discoidin Domain Receptor 2

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ABSTRACT

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Discoidin domain receptor 2 (DDR2) is one of receptor tyrosine kinase (RTKs) which has fibrillar collagens as its ligand. Fibrillar collagen interacts with pre-formed DDR2 dimers to induce dimer oligomerization and phosphorylation of the tyrosine kinase domain. The phosphorylation begins hours after the activation and persist up to 18 h.

DDR2 plays role in wound healing. As collagen receptor, it controls the matrix metalloproteinase (MMP) expression and activity to control extracellular matrix (ECM) remodeling. MMP-2 is expressed by fibroblasts and endothelial cells in acute wounds which accelerates cell migration. Moreover, DDR2 knockout fibroblasts expressed lower mRNA levels of MMP2. MMP-9 is also expressed in injured epithelia and has a role in wound healing. These data suggest the role of DDR2 with MMP-2 and MMP-9

expression during wound closure. Mitogen-activated protein kinases (MAPKs) connect extracellular signals to specific transcription factors. MMP-2 expression has been showed to be correlated with ERK1/2 and p38 kinase pathway. On top of that, p38 kinase pathway regulates MMP-9 and cell migration also.

C1q is part of the first component of complement which activates the classical pathway. It is composed by collagen-like domain and globular domain. Study proved that C1q was deposited in wound area but not detected in intact skin. In addition, topical application of C1q promotes the wound closure suggesting C1q role in wound healing.

This study aim is to investigate the binding between DDR2 with collagen-like molecule, C1q. We found that C1q binds to and phosphorylate DDR2. The phenomenon is followed by increase cell migration ability after C1q treatment by activation of MMP-2 and MMP9. We also discovered C1q increased the phosphorylation of p38 kinase and ERK1/2. These results reveal C1q as the new ligand of DDR2 which promotes the wound healing ability of epithelial cells.

Key Words: discoidin domain receptor 2, C1q, collagen

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I. INTRODUCTION

Discoidin domain receptor 2 (DDR2) is one of receptor tyrosine kinases (RTK) based on the presence of a catalytic kinase domain[1]. Structurally, it contains an N-terminal discoidin homology (DS) domain and DS-like domain which binds to collagen in their extracellular regions[2]. Further, it composed of a juxtamembrane (JM) domain and the catalytic tyrosine kinase domain (KD) ended by a short C-terminal that undergoes phosphorylation and activates downstream signaling.

Commonly, RTKs are activated by soluble peptide-like growth factors. However, DDR2 is activated by collagens which are the major components of all types of extracellular matrix (ECM)[3]. In contrast with DDR1, DDR2 is highly activated only by fibrillar collagen[2]. DDR2 is a ligand-independent dimers that form in the interaction by their transmembrane domains. Fibrillar collagen interacts with pre-formed DDR2 dimers to induce not only dimer oligomerization but also conformational changes that result in receptor

activation[4].

Unlike the interaction of other RTKs, DDR2 has a slow receptor tyrosine autophosphorylation[2]. It requires hours to reach full activation and can persist up to 18 h. Specific tyrosine residues in the activation loop of DDR2 tyrosine kinase domain are phosphorylated[5]. Collagen-stimulated DDR2 expressing cells showed that Tyr736 and Tyr741 become phosphorylated from 8 to 24 h[6]. Both Tyr736 and Tyr741 are only phosphorylated when DDR2 reaches the maximal kinase activity.

Similar to other RTKs, DDR2 is important to regulate cellular processes including cell proliferation, differentiation and survival[7, 8, 9]. DDR2 are principally functioning as sensors for ECMs. It controls the matrix metalloproteinase (MMP) expression and activity to control ECM remodeling[10]. As a whole, DDR2 plays role in wound healing[11], angiogenesis[12], skeletal growth and tumor progression[13].

In tissue regeneration and healing, collagen receptors regulate fibroblast responses to further activate downstream signaling that drives fibroblasts recruitment to the wounded area. $DDR2^{-/-}$ mice skin wounds showed delayed closure of dermal wounds[11]. Further, $DDR2^{-/-}$ skin fibroblasts express lower mRNA levels of MMP2 compared to wild type[14]. ECM remodeling also corresponds with basement membrane degrading proteases production (MMP-9) which are important in re-epithelialization[15]. These findings suggest the role of DDR2 with MMP expression during wound closure.

Mitogen-activated protein kinases (MAPKs) are parts of kinase cascade that connect extracellular signals to specific transcription factors which results in cellular responses[16]. The MAPK signaling also involved in the cellular

migration of various cell types[17]. ERK1/2 and p38 kinase pathways showed a correlation with MMP-2 expression[18]. Moreover, regulation of MMP-9 and cell migration has been proved to be through the p38 kinase pathways[19].

C1q is a collagen-like, composed by complex hexameric glycoprotein[20]. This molecule contains two major functional domains which are the collagen-like domain and the globular domain. Six globular heads are connected by six collagen-like stalks to a fibril-like central region. The molecule consists of 3 very similar but distinct polypeptide chains, A, B, and C[21]. The chains are arranged to form 6 triple helical strands, three peptide chains, into one strand.

C1q is part of the first component of complement which serves as a recognition signal triggering activation of the classical pathway[22]. Nonetheless, C1q role is not limited to recognition of immune complexes that activate the classical pathway. Cell surface receptors interact with C1q to induce stimulation of fibroblasts and endothelial cell adhesion[23], trophoblast cell migration[24], and angiogenesis[25]. Study suggests C1q was deposited in wound area but undetectable in intact skin[25]. Moreover, topical application of purified C1q promotes angiogenesis on mice. These studies indicate C1q role in wound healing but the underlying mechanism remain unclear.

In this study, we aimed to investigate the binding between DDR2 and C1q. We also investigate the C1q-mediated *in vitro* wound healing ability, using HT1080 cells as model. Underlying signaling pathways involved in the molecular mechanism of the phenomenon were also examined. We observed that C1q binds to DDR2 which leads to DDR2 phosphorylation. Furthermore, the event induces cell migration through p38 kinase and ERK1/2 pathways followed by MMP-2 and MMP-9 expression.

II. MATERIALS AND METHODS

1. Cell Cultures

The human fibrosarcoma cell line, HT1080 cells, was cultured in Minimum Essential Medium (Wellgene, Gyeongsanbuk, Korea) containing 10% fetal bovine serum and 1% penicillin-streptomycin. Cell cultures were maintained at 37^{o}C, in humidified atmosphere of 5% CO_2 in an incubator.

2. Fluorescence-activated Cell Sorting (FACS)

HT1080 cells were harvested and washed twice with Dulbecco's phosphate-buffered saline (Wellgene) then centrifuged at 6000 rpm for 10 min. Cells were fixed with 4% paraformaldehyde in RT for 30 min and washed with DPBS twice. The fixed cells were stained with or without rabbit anti-DDR2 (Santa Cruz, Texas, USA) and goat anti-rabbit alexa 488 (Invitrogen, Oregon, USA) for 1 hr at 4^{o}C in the dark. Cells were washed with DPBS and resuspend with 300 μl. All stained cell samples were analyzed by flow cytometry using FACSVerse system (BD Bioscience, San Jose, CA, USA).

3. Ensyme-linked Immunosorbent Assay (ELISA)

DDR2 (R&D Systems, Minneapolis, USA) or C1q (Sigma, Darmstandt, Germany) protein was coated on ELISA plate (Corning, NY, USA) at 4^{o}C for overnight. Plates were washed twice with DPBS

and blocked with 200 μl of 3% bovine serum albumin (Sigma) in RT for 1 hr. Wells were washed with DPBS and DDR2 or C1q protein was added and incubated at 37°C for 2 hr. Wells were washed three times and 100 μl of mouse anti-DDR2 (R&D system) or rabbit anti-C1q antibody (Dako Denmark A/S, Glostrup, Denmark) were added (1:5000 dilution in 3% BSA) for 1 hr at RT. Wells were washed twice with DPBS and incubated with horseradish peroxidase (HRP)-linked anti-rabbit (Life Technologies, Inc., Gaithersburg, USA) or anti-mouse (Life Technologies) secondary antibody for 1 hr. TMB solution (Invitrogen Corporation, CA, USA) was added for 30 min at RT. Then, the reaction was stopped using 50 μl/well of 1 N hydrogen chloride (Duksan Pure Chemicals Co., Ansan, Korea). Plates were analyzed in an ELISA reader (Molecular Devices, CA, USA) at 450 nm, buffer only-values were subtracted and normalized values were subjected to statistically analysis.

4. Co-immunoprecipitation Assay

DDR2 and C1q protein was mixed in the same tube and incubated at 37°C for 2 hr. Complete His-Tag Purification Resin (Roche Diagnostics GmbH, Mannheim, Germany) was blocked with 3% BSA (Sigma) in 4°C for 1 hr. Then, the resin was mixed with the proteins and the mixtures were shaken on rotating shaker at 4°C overnight. The supernatant was obtained and used in the immunoblotting assay.

5. Immunofluoresence Assay

The cells were seeded and fixed in 4% paraformaldehyde for 30 min on ice and then washed three times on ice with DPBS. The cells were blocked by 3% BSA for 30 min and stained with or without mouse anti-DDR2 antibody (R&D system) at 4^{o}C for overnight and with or without rabbit anti-C1q antibody (Dako). Secondary goat anti-rabbit alexa 488 or goat anti-mouse alexa 594 antibody (Invitrogen) was given for 45 minute in the dark. The nuclei were stained with DAPI and cells were observed with a fluorescence microscope. To determine the percentage of 2 proteins colocalization, images were loaded into ImageJ software and the ratios of green or red to merged cells were measured with the colocalization plug-in.

6. Proximity Ligation Assay

Proximity ligation assay (PLA) was performed according to the manufacturer's protocol (Duolink, Sigma). The cells were washed with cold DPBS and fixed with 4% paraformaldehyde for 30 min on ice. The cells were blocked with 3% BSA for 30 min and incubated overnight at 4^{o}C with primary antibody rabbit anti-C1q antibody (Dako) and mouse anti-DDR2 antibody (R&D system). The cells then incubated with corresponding PLA probes conjugated to oligonucleotides (mouse MINUS and rabbit PLUS), followed by ligation and rolling circle amplification in close proximity. The nuclei were stained with DAPI and cells were observed with a fluorescence microscope. Interactions were quantified by counting the number of

dots per cell using ImageJ software.

7. In Vitro Cell Wound Healing Assays

The cells were grown to confluence in 12-well plates (Corning Life Science, NY, USA). A straight scratch was made using a 1 ml pipette tip. The cells were then washed with PBS twice and further cultured with collagen or C1q (20 μg/ml) for indicated hours. At the indicated time, the gap of scratch was recorded using Olympus IX73 Inverted Microscope (Olympus, Tokyo, Japan). Using the ImageJ, the size of the denuded area was determined at each time point from the digital images.

8. Gelatin Zymography

The cells were treated with collagen or C1q in media containing 2% serum. The medium was then harvested, briefly centrifuged and immediately frozen at -20^{o}C to prevent auto-activation of MMPs. Aliquots of medium were later prepared with a native buffer. Proteins were resolved by 8% polyacrylamide gels containing 0.1% gelatin without boiling. Gels were incubated in 1x Zymogram Renaturation Buffer (Bio-rad, CA, USA) for 30 min. Gels were washed with distilled water and then incubated in 1x Zymogram Development Buffer (Bio-rad) for 30 min. Then the gels were incubated with a new 1x Zymogram Development Buffer for overnight at 37 ^{o}C. The gels were then stained with 0.5% coomassie brilliant blue in 10% acetic

acid and 50% methanol. The gels were destained with 50% methanol and 10% acetic acid solution and were exposed to a MiniBIS Pro imager (DNR Bio-Imaging Systems Ltd., Neve Yamin, Israel).

9. Western Blot

The cell lines were lyzed in RIPA cell lysis buffer (GenDEPOT Inc., TX, USA) to obtain protein extracts. Protein concentration was determined by BCA protein assay kit (Pierce Biotech, IL, USA). Equal amounts of protein were resolved by 8 or 12% SDS polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to nitrocellulose membranes (Amersham Biosciences, Buckinghamshire, UK). After blocking in Tris-buffered saline containing 5% skim milk and 0.1% Tween-20, the membranes were incubated with primary antibodies, followed by incubation with HRP-labeled secondary antibodies. The addition of West-Q Pico ECL solution (GenDEPOT Inc.) was used to develop the images. The following primary antibodies were used: mouse anti-DDR2 (1:1000, R&D Systems), rabbit anti-DDR2 (1:1000, Santa Cruz), rabbit anti-C1q (1:500, Dako), mouse anti-phosphotyrosine (1:1000, Merck Milipore, Massachusetts, USA), rabbit anti-phospho DDR2 (1:1000, R&D systems), rabbit anti-pp38 (1:1000, Cell Signaling Technology, Danvers, MA, USA), rabbit anti-p38 (1:1000, Cell Signaling Technology), rabbit anti-pERK1/2 (1:1000, Cell Signaling Technology), rabbit anti-ERK1/2 (1:1000, Cell Signaling Technology), rabbit anti-β-actin (1:5000, Cell Signaling Technology) antibody.

10. Short Hairpin RNA and Transfection Assay

DDR2 knockdown using stable short-hairpin interfering RNAs. For targeting DDR2 (NM_006182 NCBI), we used #TRCN0000001418 from Yonsei Genomics Center System Biology Core. #SHC016 was also used as scramble sequence shRNA. HT1080 cells were transfected using Fugene HD transfection reagent (Roche Diagnostics) according to the manufacturers' instruction. The transfected cells were further identified by western blot. After selection and identification, the same method of transfection was done for wound healing assay and gelatin zymography assay.

11. Statistical Analysis

Results are presented as the mean and standard deviation (SD). Comparisons between two groups were performed using an unpaired two-sided Student's t-test. Data were analyzed using the IBM SPSS Statistics version 22.0 program (International Business Machines Corp., New York, USA). A p-value < 0.05 was considered statistically significant.

III. RESULTS

1. Binding of C1q to DDR2

Previous studies have shown that DDR2 is activated by fibrillar collagens, in particular collagen type I[26]. In this study, we proved the binding through ELISA assay and use the result as our positive control (Fig. 1A). C1q which has a collagen-like structure was expected to bind to DDR2. In order to prove the hypothesis, microtiter wells were coated with 8 μg/ml DDR2 and C1q protein was added to the wells and the binding was examined by ELISA. The results showed C1q bound to DDR2 in a concentration-dependent manner (Fig. 1B). In the reverse experiment of DDR2 binding to coated C1q showed the similar result (Fig. 1C).

To detect the interaction between C1q and DDR2, we also performed a co-immunoprecipitation analysis by mixing equal concentrations (100 ng/ml) of C1q and His-tag fused DDR2 protein. Pull down was done with his-tag sensitive resin which interacted to the DDR2 protein. We analyzed the bound proteins by western blot using antibodies specific for C1q and DDR2, respectively. Two clear bands were confirmed corresponding to the size of C1q by antibodies against C1q (Fig. 1D). DDR2 bands were also confirmed as shown in the western blot result, suggesting the significant binding of C1q to DDR2.

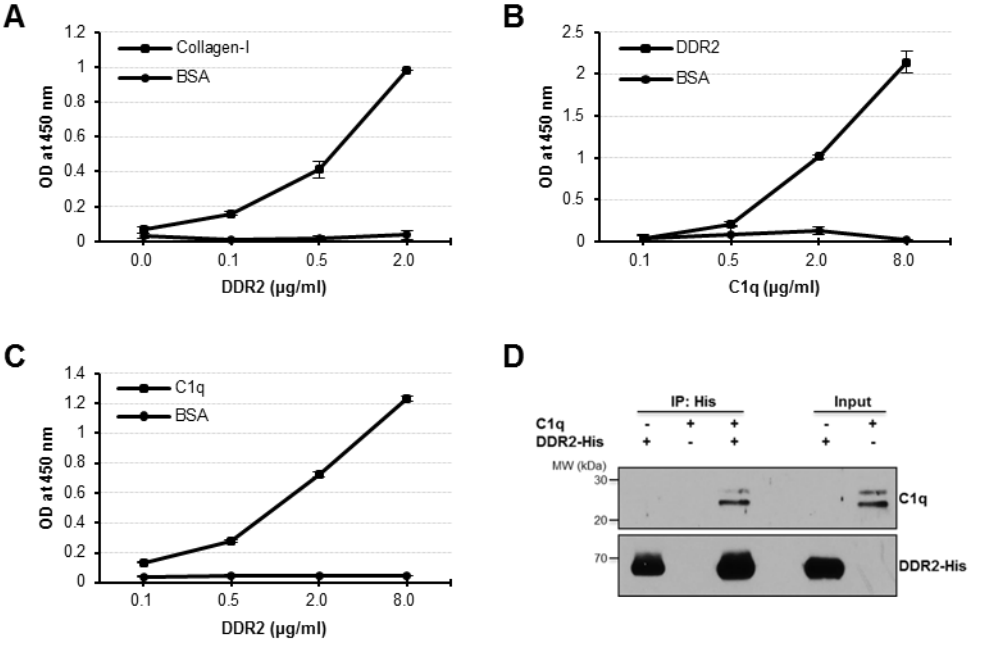
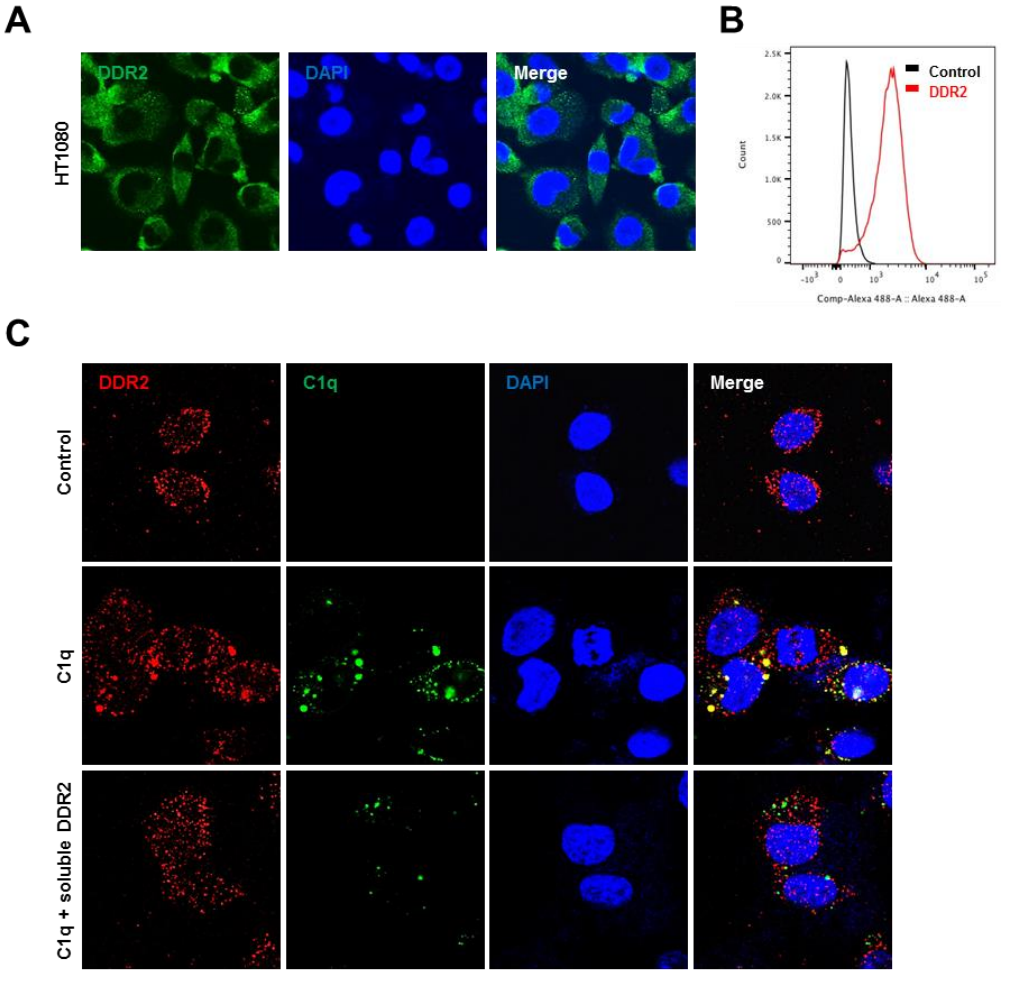


Figure 1. C1q Binds to DDR2. (A) Collagen (2 μg/ml) was immobilized in wells of microtiter plates; various concentrations of DDR2 were added and binding was evaluated by ELISA. This result was used as a positive control. (B,C) Either DDR2 or C1q (8 μg/ml) was immobilized in wells of microtiter plate; various concentration of C1q (B) or DDR2 (C) were added and binding was evaluated by ELISA (OD = Optical Density). (D) Co-immunoprecipitation of DDR2 and C1q. C1q was incubated with DDR2 for 2 h at 37^{o}C followed by capture with sepharose-based complete his-tag purification resin. Antibodies for western blot analyses are indicated.

2. C1q Binds to DDR2 Expressed by HT1080

DDR2 is well known for its function in wound healing[11,14,27]. In vitro experiments using DDR2$^{-/-}$ skin fibroblast demonstrated that skin wounds healed more slowly in DDR2$^{-/-}$ mice than in wild-type ones[11]. Other study also showed that C1q promotes wound healing[25]. We suggest that C1q promotes wound healing by binding to DDR2. In order to prove the statement, HT1080 which is epithelial originated cell line were used. DDR2 was highly expressed in the HT1080 cell line by immunofluorecence assay (Fig. 2A) and Fluorescence-activated cell sorting (Fig. 2B).

To investigate whether C1q is able to bind to DDR2 expressed by the cell line, we observed the colocalization of C1q and DDR2 using confocal microscopy. The results showed that C1q and DDR2 largely overlap with each other (Fig. 2C). We also confirm the binding using *in situ* proximity ligation assay. The cellular protein-protein interactions *in situ* were confirmed (Fig. 2D). The specificities of these interactions were further demonstrated by blocking experiments, in which a soluble DDR2 protein inhibited binding of C1q to DDR2 expressed on the cell line.



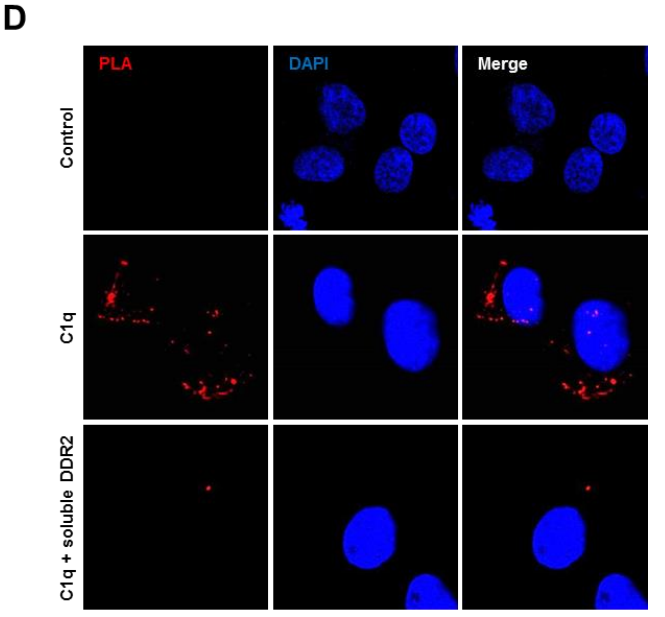


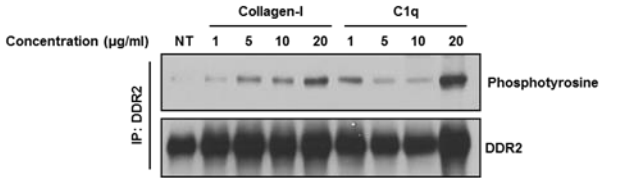
Figure 2. C1q Binds to DDR2 Expressed by HT1080. (A) Confocal images from immuno-staining of HT1080 cells for DDR2. (B) DDR2 expression analyzed by flow cytometry. Red histograms represent staining with DDR2 antibody; black histograms represent the isotype control, in HT1080 cells. (C) Colocalization of DDR2 and C1q. The cells were pre-treated with or without 2 μg/ml of soluble DDR2 following by treatment with or without C1q (2 μg/ml). Confocal images of the cells showing dual staining for DDR2 (red) with C1q (green). (D) Proximity ligation assay showing specific interactions of C1q and endogenous DDR2 (red spots). The cells were treated similar to Fig. 2C.

3. DDR2 Phosphorylation by C1q Promotes Wound Healing

DDR2 activation is known to be delayed and sustained for over 24 h[6]. To identify the phosphorylation-mediated signaling networks downstream of DDR2 activation by C1q, HT1080 cells were stimulated with several designated concentration of C1q for 24 h. Addition of C1q to cells resulted in DDR2 phosphorylation, as observed by receptor immunoprecipitation with anti-DDR2 followed by immunoblot analysis for phosphotyrosine (Fig. 3A).

The effect of C1q on DDR2 wound healing function was analyzed using a scratch assay (Fig. 3B-C). Confluent monolayers of cells were wounded with a uniform scratch, washed to remove debris and incubated in the absence or presence of C1q for the designated time point. The result of the scratch assay revealed that the wound closure level, which corresponds to the wound healing ability, was significantly increased by C1q (20 μg/ml) treatment compared with the untreated control cells. Collagen treated cells were used as positive control.

A



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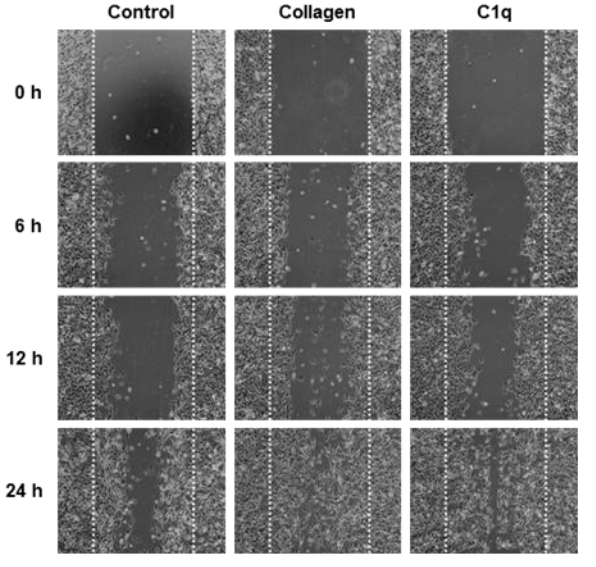

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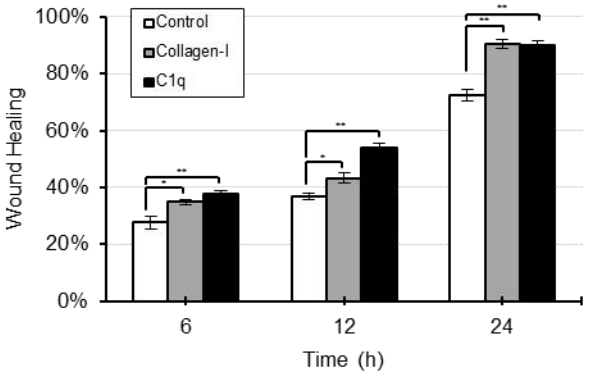

Figure 3. C1q Promotes DDR2 Phosphorylation and Its Wound Healing Function. (A) Immunoprecipitation and western blotting for DDR2 and tyrosine phosphorylation were performed in HT1080 cell line. After stimulation with collagen or C1q for 24 h with the indicated concentration, an expression band corresponding to phosphorylated DDR2 was observed. (B) Effects of C1q on the wound healing ability of HT1080 cells. Optical microscopic images of in vitro wound healing at indicated hours after creation of wounds. Collagen (20 μg/ml) treated cells were used as positive control. (C) The wound areas of HT1080 cells were calculated and expressed as the percentage of wound closure to the initial wound areas. Values are presented as means ± SD of three independent experiments. *, $p < 0.01$. **, $p < 0.001$.

4. C1q Induces MMP-2 and MMP-9 Expression via p38 and ERK1/2 pathways

Matrix metalloproteinases (MMPs) have a key role in wound healing by modifying the wound matrix, allowing for cell migration and tissue remodeling[28]. MMP-2 is expressed by fibroblasts and endothelial cells in human acute wounds. Some studies proved that MMP-2 not only accelerates cell migration[29] but also mediates platelet adhesion and aggregation[30]. Likewise, MMP-9 is also expressed in several injured epithelia and has a role in wound healing[31].

In order to determine the effect of C1q on MMP-2 and MMP-9 enzyme activities, gelatin zymography was performed (Fig. 4A). We confirmed that C1q induces MMP-2 and MMP-9 expression in a time-dependent manner. The active bands of MMP-2 and MMP-9 gradually increased when cells were treated with increasing treatment time of C1q (Fig. 4B-C). Collagen treated samples were used as positive control.

Studies have indicated the correlation between p38 kinase and ERK1/2 pathways with MMP-2 and MMP-9 activity[18, 19]. To assess whether C1q regulates the pathways, we investigate the phosphorylation status of p38 and ERK1/2 in HT1080 cells after treatment with 20 μg/ml C1q at different time periods (Fig. 4D). As shown in the Fig. 4D, C1q significantly increased the phosphorylation of p38 and ERK1/2 in a time-dependent manner..

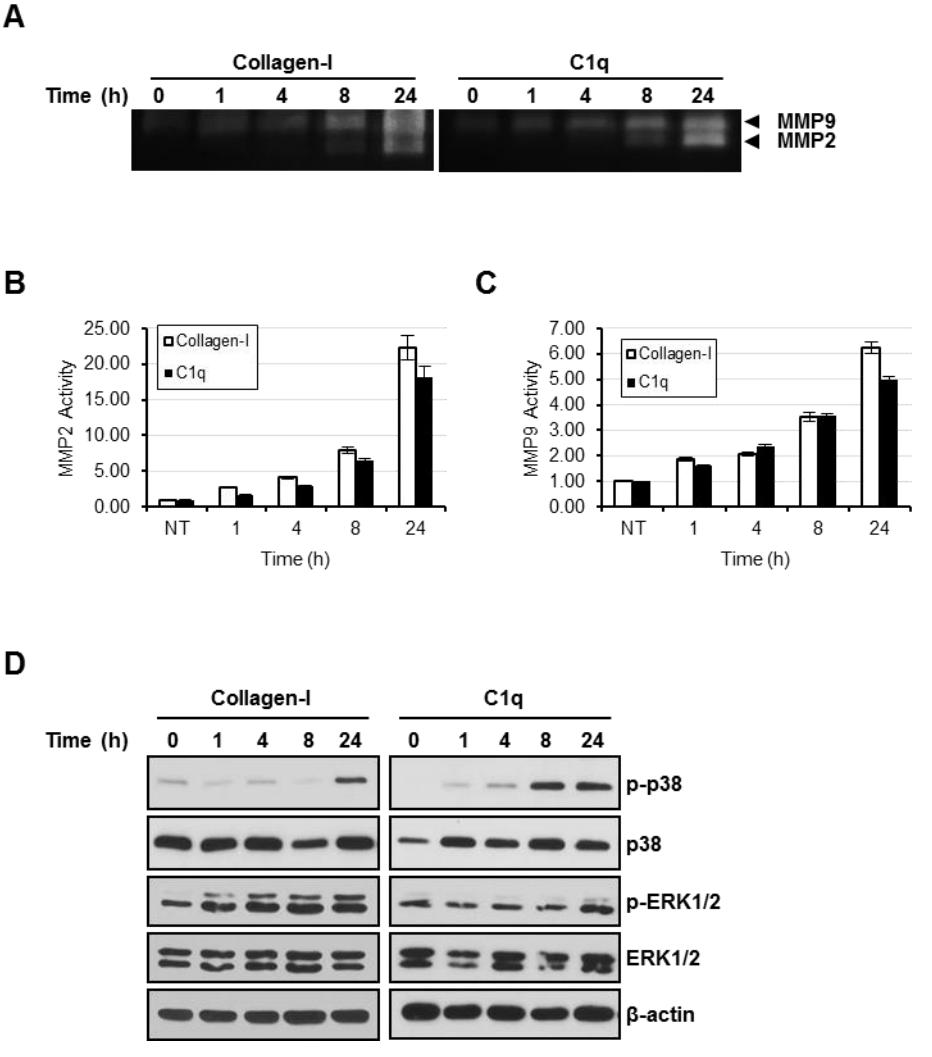

Figure 4. C1q Induces MMP2 and MMP9 Expression via p38 and ERK1/2. (A) Gelatin zymograph of HT1080 cell lines. The cells were treated with collagen-I or C1q (20 μg/ml). Then, the cell supernatants were subjected to SDS-PAGE zymography to assess the content of MMP-2 and MMP-9 derived gelatinase activity. (B,C) Zymographic band densities from all samples were quantified by ImageJ. The relative enzyme activities of MMP-2 (B) and MMP-9 (C) are expressed as a mean ± SD of three independent experiments. (D) HT1080 cells were treated with collagen or C1q (20 μg/ml) for the indicate time periods. The expressions of p-p38, p38, pERK1/2, ERK1/2 were detected using western blot analysis.

5. Knockdown of DDR2 with shRNA Attenuated Wound Healing

To determine DDR2 significant role in C1q wound healing effect, DDR2 was transiently knock down using short hairpin RNAs (shRNA) in HT1080 cell line. The knockdown result was confirmed using western blot (Fig. 5A). DDR2 expression in HT1080 cells was abolished with shDDR2.

The same plasmids then assessed on *in vitro* scratch test (Fig. 5B). Deletion of DDR2 abolished C1q-induced cell migration (Fig. 5C). As shown in the result, when DDR2 was silenced the cell migration ability was depleted. Addition of C1q on the knock down cells slightly rescued the phenomenon. Following knockdown of DDR2 and C1q treatment together, cell migration ability decreased by ~23% compared to C1q treatment alone. This result suggests the important role of DDR2 in C1q-induced cell migration.

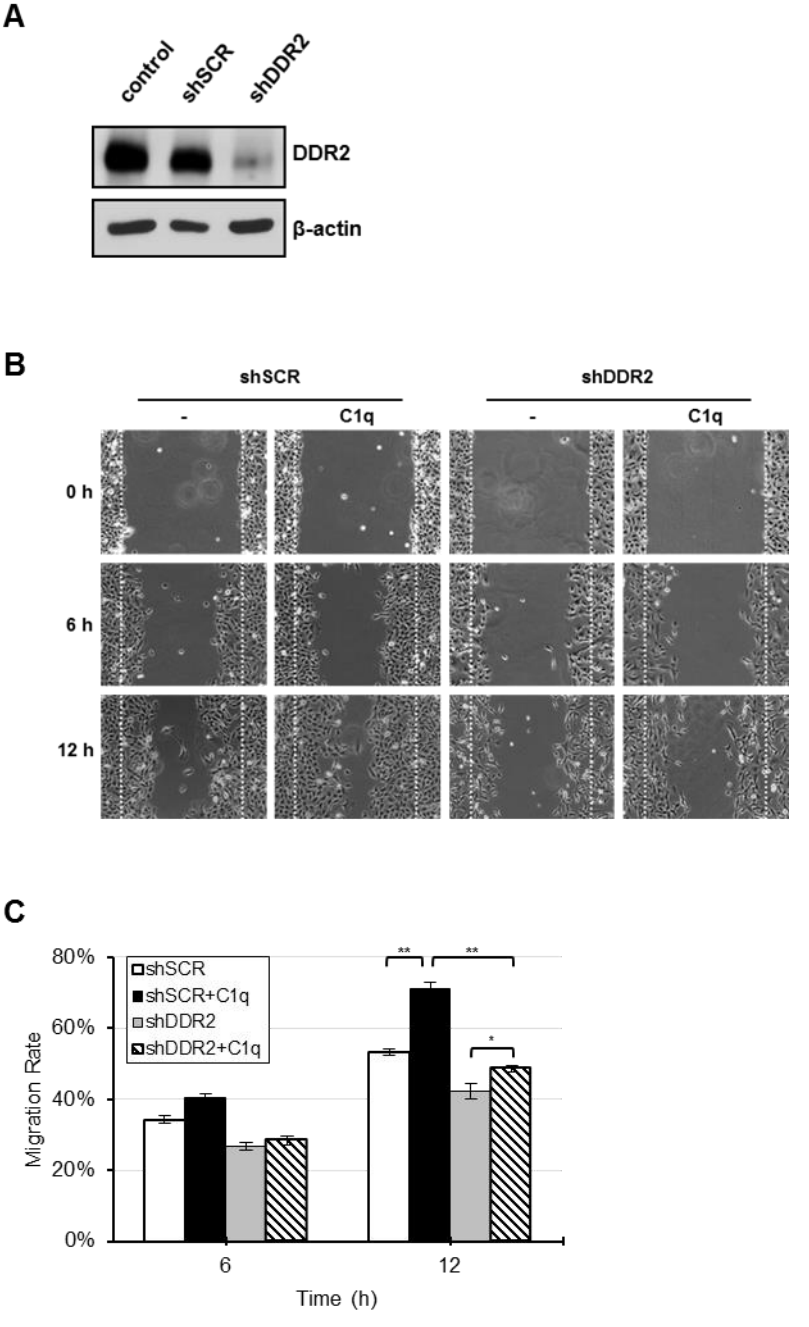

Figure 5. Silencing DDR2 Inhibits C1q Wound Healing Effect. (A) Knock down of DDR2 in HT1080 cells. Western blotting was performed to detect the presence of DDR2 in the cells after transiently transfected with DDR2 shRNA, while the scrambled shRNA was used as the control vector. (B) The wound healing assay was performed with HT1080 cells as described in Fig. 3B using DDR2 shRNA or scrambled shRNA transfection. After the transfection, the cells were treated with or without C1q (20 μg/ml) (C) The wound areas of HT1080 were calculated and expressed as the percentage of wound closure to the initial wound areas. Values are presented as means ± SD of three independent experiments. *, $p < 0.05$. **, $p < 0.01$.

IV. DISCUSSION

C1q is a molecule which contains collagen-like domain. Its main role is to recognize signals and activate the classical pathway[22]. C1q also interacts with protein on the cell surface to conduct cellular responses[32]. $C1qR_p$ is known to be the major C1q receptor though it has two other ligands which are MBL and SPA. Other molecules also have been identified as C1q receptors which is cC1qR and gC1qR[33]. However, both protein existences on the surface are dependent to stress or apoptotic environment. Additionally, C1q also has several ligands such as IgG, IgM, laminin, fibronectin, amyloid Aß peptide, and fibromodulin which may cause complement dysregulation and lead to disease[34]. Not only that, C1q also binds to LAIR-1 and mediates immunoinhibitory activity[35].

The discoidin domain receptors are activated by collagen, major components of ECM[3]. While the non-fibrillar collagens are ligands for DDR1, DDR2 is known to be activated by fibrillar collagen in particular collagen type I[2]. The DDRs then undergo ligand-induced phosphorylation in a slow manner. This study found that C1q is also a ligand for DDR2. Our data may open possibilities for other collagen-like containing molecules such as mannose-binding lectin, ficolin, pentraxin and adiponectin to be the ligand for DDRs.

The DDRs are important in development. DDR1 is important in organogenesis as its knockout mice show defects in kidney and inner ear architecture[36]. Also, the mice displayed abnormal secondary branching[37]. On the other hand, DDR2 plays role in bone growth. DDR2 deletion in mice resulted in dwarfism with short long bones and reduced chondrocyte proliferation[38]. The mice were named *slie* due to their sterile phenotype with the female were anovulatory and the males lacked spermatogenesis[27].

Despite of their developmental function, mutations and altered expression of DDRs are found in lung and liver fibrosis[39, 40], atherosclerosis[41], osteoarthritis[42], and many types of cancers[43]. Moreover, as collagen receptors they mediate fibroblast responses during tissue generation and healing. DDR2 interact with collagenous ECM and its mRNA is also up-regulated in dermal burn wounds[44]. In addition, DDR2 knockout mice showed delayed closure of dermal wound[11]. Hence, DDR2 has an important role in wound healing.

C1q is detected in the lesion area of dermal wound[25]. It was mainly localized in the surrounding stroma and in scattered inflammatory cells. The protein functions at the lesion area are to stimulate endothelial proliferation and migration, and to promote angiogenesis. However, the C1q-induced wound healing mechanism is still unknown.

In this study we found that C1q is a ligand to DDR2 and both have a role in wound healing. So, we suggest that C1q-mediated wound healing ability is through binding with DDR2. In order to prove the hypothesis, HT1080 cells were used in this study. The cell line is epithelial cell line which has been well known to be used for *in vitro* wound healing assay. The result of this study is that C1q promotes the migratory ability of the cells.

As mentioned before, C1q has several cell surface protein receptors. To identify the significant role of DDR2 in C1q-mediated wound healing ability, DDR2 was knocked down in HT1080 cells and cells were then treated with or without C1q. Deleting DDR2 resulted in delayed cell migration. This result suggests that C1q binds to cell membrane bound DDR2 to regulate epithelial cell migration.

In conclusion, these data suggest C1q as the new ligand of DDR2. C1q

binding to DDR2 results in phosphorylation of DDR2 and increase the migratory ability in HT1080 cells. C1q increase not only the activity of MMP-2 and MMP-9 but also the phosphorylation of p38 and ERK1/2. Despite the fact that the exact mechanism remains unclear, the present study provides a base for future experiments on C1q functions as the ligand of DDR2.

Our study also proved that there are possibilities for DDRs to bind with other collagen-like containing molecules. Further study about the new ligands of DDRs may result in advance understanding of DDR-mediated physiological phenomenons and discovery to new therapies for DDR-mediated disease.

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V. CONCLUSION

This study demonstrated that fibrillar collagen is not the only ligand to DDR2. The receptor also binds to collagen-like molecule, C1q. Following the phosphorylation of DDR2, C1q promotes the epithelial cell migratory ability. The downstream signals of this phenomenon include activation of MMP-2 and MMP-9 and phosphorylation of p38 and ERK1/2. Collectively these data reveal a novel finding in new ligand of DDR2 which is a component of innate immunity, C1q.

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ABSTRACT (IN KOREAN)

DDR2 리간드로써 C1q의 역할과 기능

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Discoidin domain receptor2 (DDR2)는 타이로신 인산화효소 수용체 중 하나로서 미소 섬유의 콜라겐을 리간드로 가진다. 미소 섬유 콜라겐은 DDR2 이량체의 전 형태와 상호작용하는데 이로 인해 이량체의 올리고머화와 티로신 인산화 효소 도메인의 인산화가 유도된다. 인산화는 활성화 몇 시간 후 시작되며, 이 활성화는 18시간까지 지속된다.

DDR2는 상처 회복을 담당하는데, 콜라겐 수용체로서 세포 외 기질 리모델링을 조절하는 기질금속단백질분해효소 발현과 활성을 조절한다. 기질금속단백질분해효소-2는 세포의 이동을 촉진시키는 급성 상처를 입은 섬유아세포와 내피세포에 의해서 발현된다. 또한 DDR2가 녹 아웃된 섬유아세포에서는 낮은 수준의 기질금속단백질분해효소-2 mRNA가 발현된다. 기질금속단백질분해효소-9는 또한

상처 입은 상피세포에서 발현되는데, 이는 상처회복을 담당한다. 이 논문은 상처 봉합 동안에 기질금속단백질분해효소-2, 9과 관련된 DDR2의 역할을 제시한다. 미토켄 활성화단백질 인산화효소는 세포 외부의 신호를 특정 전사 인자에 전달하는데, 기질금속단백질분해효소-2 발현은 ERK1/2와 p38 인산화 효소 경로와 관련 있다는 것이 밝혀졌고, p38 인산화 효소 경로는 기질금속단백질분해효소-9와 세포 이동을 조절함 역시 보고된 바 있다.

C1q는 고전 경로를 활성화시키는 보체의 첫 번째 구성요소로, 콜라겐과 유사한 도메인 (collagen-like domain)과 공 모양 (globular domain)의 도메인으로 구성된다. 이번 연구에서는 C1q가 상처구역에 위치하지만 정상 피부에서는 탐지되지 않음을 증명하였다. 그리고 C1q를 국소 처리하면 상처봉합이 촉진되는데, 이는 C1q가 상처 회복에 기여함을 시사한다.

이 연구의 목표는 DDR2가 콜라겐과 비슷한 분자인, C1q와 결합 여부를 밝히는 것이다. 우리는 C1q가 인산화된 DDR2와 결합함과 동시에, C1q 처리에 의해 기질금속단백질분해효소-2, -9가 활성화되어 세포 이동이 증가하는 현상을 볼 수 있었다. 또한 C1q가 p38 인산화 효소와 ERK1/2의 인산화를 증가시킴을 발견하였다. 이러한 결과들은 C1q가 상피세포의 상처회복 능력을 촉진시키는 DDR2의 새로운 리간드임을 증명한다.

핵심되는 말 : discoidin domain receptor2 (DDR2), C1q, 콜라겐